

# **EXHIBIT 49**

## Final Report on the Safety Assessment of Sodium *p*-Chloro-*m*-Cresol, *p*-Chloro-*m*-Cresol, Chlorothymol, Mixed Cresols, *m*-Cresol, *o*-Cresol, *p*-Cresol, Isopropyl Cresols, Thymol, *o*-Cymen-5-ol, and Carvacrol<sup>1</sup>

Sodium *p*-Chloro-*m*-Cresol, *p*-Chloro-*m*-Cresol (PCMC), Mixed Cresols, *m*-Cresol, *o*-Cresol, *p*-Cresol, Isopropyl Cresols, Thymol, Chlorothymol, *o*-Cymen-5-ol, and Carvacrol are substituted phenols used as cosmetic biocides/preservatives and/or fragrance ingredients. Only PCMC, Thymol, and *o*-Cymen-5-ol are reported to be in current use, with the highest concentration of use at 0.5% for *o*-Cymen-5-ol in perfumes. The use of PCMC in cosmetics is restricted in Europe and Japan. Cresols can be absorbed through skin, the respiratory tract, and the digestive tract; metabolized by the liver; and excreted by the kidney as glucuronide and sulfate metabolites. Several of these cresols increase the dermal penetration of other agents, including azidothymidine. In acute oral toxicity studies, LD<sub>50</sub> values were in the 200 to 5000 mg/kg day<sup>-1</sup> range across several species. In short-term studies in rats and mice, an *o*-Cresol, *m*-Cresol, *p*-Cresol or *m*-Cresol/*p*-Cresol mixture at 30,000 ppm in the diet produced increases in liver and kidney weights, deficits in liver function, bone marrow hypocellularity, irritation to the gastrointestinal tract and nasal epithelia, and atrophy of female reproductive organs. The no observed effect levels (NOEL) of *o*-Cresol was 240 mg/kg in mink and 778 mg/kg in ferrets in short-term feeding studies, with no significant dose-related toxicity (excluding body weight parameters). In mice, 0.5% *p*-Cresol, but neither *m*-Cresol nor *o*-Cresol, caused loss of pigmentation. Short-term and subchronic oral toxicity tests performed with various cresols using mice, rats, hamsters, and rabbits resulted in no observed adverse effect levels (NOAELs) for mice of 625 ppm and rats of 50 mg/kg day<sup>-1</sup>, although the NOEL was 2000 ppm in a chronic study using rats. In rabbits, ≤160 mg/kg PCMC was found to produce irritation and erythema, but no systemic effects. Hamsters dosed with 1.5% *p*-Cresol in diet for 20 weeks had a greater incidence of mild and moderate forestomach hyperplasia as compared to the control. Acute inhalation toxicity studies using rats yielded LC<sub>50</sub> values ranging from >20 mg/m<sup>3</sup> for *o*-Cresol to >583 mg/m<sup>3</sup> for PCMC. No deaths were recorded in mice given *o*-Cresol at 50 mg/m<sup>3</sup>. Cats exposed (short-term) to 9 to 50 mg/m<sup>3</sup> of *o*-Cresol developed inflammation and irritation of the upper respiratory tract, pulmonary edema, and hemorrhage and perivascular sclerosis in the lungs. Rats exposed (subchronic) to *o*-Cresol at 9 mg/m<sup>3</sup> had changes in leukocytes, spinal cord smears, nervous activity, liver function, blood effects, clinical signs, and

neurological effects. In guinea pigs, exposure to 9 mg/m<sup>3</sup> produced changes in hemoglobin concentrations and electrocardiograms (EKGs). Rats exposed (subchronic) to 0.05 mg/m<sup>3</sup> Mixed Cresols by inhalation exhibited central nervous system (CNS) excitation, denaturation of lung protein, and decreased weight gain. All cresols appear to be ocular irritants. Numerous sensitization studies have been reported and most positive reactions were seen with higher concentrations of Cresol ingredients. Developmental toxicity is seen in studies of *m*-Cresol, *o*-Cresol, and *p*-Cresol, but only at maternally toxic levels. In a reproductive toxicity study of a mixture of *m*-Cresol and *p*-Cresol using mice under a continuous breeding protocol, 1.0% caused minimal adult reproductive and significant postnatal toxicity in the presence of systemic maternal toxicity. The *o*-Cresol NOAEL was 0.2% for both reproductive and general toxicity in both generations. Cresol ingredients were generally nongenotoxic in bacterial, fruit fly, and mammalian cell assays. Thymol did not induce primary lung tumors in mice. No skin tumors were found in mice exposed dermally to *m*-Cresol, *o*-Cresol, or *p*-Cresol for 12 weeks. In the trypan blue exclusion assay, antitumor effects were observed for Thymol and Carvacrol. Clinical patch testing with 2% PCMC may produce irritant reactions, particularly in people with multiple patch test reactions, that are misinterpreted as allergic responses. *o*-Cresol, *p*-Cresol, Thymol, Carvacrol, and *o*-Cymen-5-ol caused no dermal irritation at or above use concentrations. In two predictive patch tests, PCMC did not produce a sensitization reaction. Overall, these ingredients are not significant sensitizing or photosensitizing agents. The Cosmetic Ingredient Review (CIR) Expert Panel noted some of these ingredients may increase the penetration of other cosmetic ingredients and advised cosmetic formulators to take this into consideration. The CIR Expert Panel concluded that the toxic effects of these ingredients are observed at doses higher than would be available from cosmetics. A concentration limitation of 0.5% was chosen to ensure the absence of a chemical leukoderma effect. For *p*-Cresol and Mixed Cresols (which contain *p*-Cresol), the Panel considered that the available data are insufficient to support the safety of these two ingredients in cosmetics. Studies that would demonstrate no chemical leukoderma at concentrations of use of *p*-Cresol and Mixed Cresols, or would demonstrate a dose response from which a safe concentration could be derived, are needed.

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<sup>1</sup>Reviewed by the Cosmetic Ingredient Review (CIR) Expert Panel. Report prepared by Alex Escobar former CIR staff person.

### INTRODUCTION

Cresols are methylphenol compounds, which are naturally occurring or synthetic, many of which are used as cosmetic biocides/preservatives in cosmetics. This assessment considers

the safety of the following group of Cresol ingredients in cosmetics:

Sodium *p*-Chloro-*m*-Cresol (CAS no. 15733-22-9),  
*p*-Chloro-*m*-Cresol (CAS no. 59-50-7),  
Chlorothymol (CAS no. 89-68-9),  
Mixed Cresols (CAS no. 1319-77-3),  
*m*-Cresol (CAS no. 108-39-4),  
*o*-Cresol (CAS no. 95-48-7),  
*p*-Cresol (CAS no. 106-44-5),  
Isopropyl Cresols (no CAS no.),  
*o*-Cymen-5-ol (an isopropyl cresol, CAS no. 3228-02-2),  
Thymol (an isopropyl cresol, CAS no. 89-83-8), and  
Carvacrol (also an isopropyl cresol, CAS no. 499-75-2).

The safety of Cresols was reviewed by the World Health Organization (WHO) in 1995. The WHO report concluded that “there is clear evidence in humans that, during dermal or oral exposure, high concentrations of Cresols are corrosive, absorbed rapidly and produce severe toxicity that may result in death. Inhalation may result in irritation of the respiratory tract. There is no information regarding the chronic toxicity of Cresols and no adequate data regarding the carcinogenic potential of these compounds.” The WHO report also expressed a need for studies on the toxic mechanisms of Cresols and workers occupationally exposed to Cresols (WHO 1995).

Carvacrol is not currently listed in the *International Cosmetic Ingredient Dictionary and Handbook* as a cosmetic ingredient and normally would not be included, but it is an Isopropyl Cresol, which is listed as a cosmetic ingredient, so Carvacrol was included.

In 1984, the Cosmetic Ingredient Review (CIR) Expert Panel completed a safety assessment of *o*-Cymen-5-ol (also an Isopropyl Cresol), and, in 1997, a safety assessment of *p*-Chloro-*m*-Cresol. In both cases the conclusion was that the available data were insufficient to support the safety of these ingredients in cosmetic formulations. In 1993, additional data were provided for *o*-Cymen-5-ol. Although several data needs were met, the data were still insufficient. *o*-Cymen-5-ol and *p*-Chloro-*m*-Cresol are structurally similar to other ingredients in this report and are included for review of their safety in cosmetics.

## CHEMISTRY

### Definition and Structure

The chemical definition and structure of the 11 ingredients in this review are presented in Table 1. Synonyms for each ingredient are given in Table 2.

### Physical and Chemical Properties

The physical and chemical properties for the ingredients are listed in Tables 3a for cresols and 3b for isopropyl cresols. No information was found regarding the physical and chemical properties of Sodium *p*-Chloro-*m*-Cresol.

*o*-Cymen-5-ol reacts readily with oxidizing agents to form methyl bridged dimers and quinone dimers (CTFA 1981).

### Method of Manufacture

Budavari (1989) described the preparation of these ingredients:

- *p*-Chloro-*m*-Cresol (PCMC) is prepared by the chlorination of *m*-cresol.
- Mixed Cresols is a mixture of isomers obtained from coal tar (from coke and gas works), toluene, or petroleum and is derived by sulfonation or oxidation.
- *m*-Cresol is derived from fractional distillation of crude Cresol (from coal tar) or synthetically (no other details).
- *o*-Cresol is derived from fractional distillation of crude Cresol (from coal tar) and interaction of methanol and phenol.
- *p*-Cresol is derived from fractional distillation of crude Cresol (from coal tar) and from benzene by the cumene process.
- Thymol is produced synthetically from *m*-Cresol and isopropyl chloride by the Friedel-Crafts method at  $-10^{\circ}\text{C}$  or naturally from thyme oil or other oils.

Thymol also can be derived naturally from thyme, savory, marjoram (Teissedre and Waterhouse 2000), cumin, and oregano (Leung 1980).

Carvacrol is derived from *p*-cymene by sulfonation followed by alkali fusion (Lewis 1997). Carvacrol can also be derived from savory (Teissedre and Waterhouse 2000; Martini et al. 1996), thyme, marjoram (Teissedre and Waterhouse 2000), oregano, lovage root, and Spanish origanum oil (Leung 1980).

According to the WHO (1995), several other processes have been developed to keep up with demand. For example *o*-Cresol can be produced by methylation of phenol in the presence of a catalyst and *p*-Cresol can be produced by toluene sulfonation followed by alkaline hydrolysis. The total production of Cresols in the USA (excluding production from coke oven and gas-retort ovens) was 38,300 tons in 1990.

Cresol and cresol derivatives occur naturally in oil of various plants such as *Yucca gloriosa* flowers, jasmine, peppermint, eucalyptus, camphor, Easter lily, conifers, oaks, and sandalwood trees and are also products of combustion from natural fires and volcanic activity (WHO 1995).

The published literature does not contain information regarding the method of manufacture for Sodium *p*-Chloro-*m*-Cresol, Chlorothymol, Isopropyl Cresols, or *o*-Cymen-5-ol.

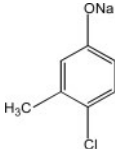
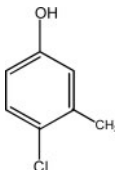
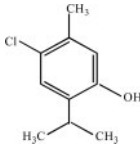
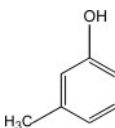
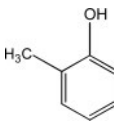
### Analytical Methods

Many analytical methods have been cited for these ingredients. This information is summarized in Table 4.

## COSMETIC INGREDIENT REVIEW


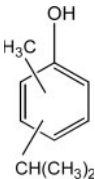
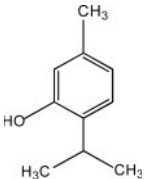
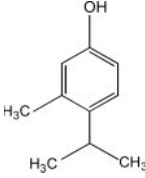
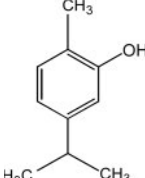
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**TABLE 1**  
Ingredient definition and structure

Ingredient	Description	Uses	Reference
Sodium <i>p</i> -Chloro- <i>m</i> -Cresol	The organic compound that conforms to the following formula and structure: $C_7H_7ClO \cdot Na$	Preservatives	Pepe et al. (2002)
			
<i>p</i> -Chloro- <i>m</i> -Cresol	<i>p</i> -Chloro- <i>m</i> -Cresol generally conforms to the structure:	Cosmetic biocides and preservatives	Pepe et al. (2002)
			
Chlorothymol	The halogenated phenol that conforms to the following formula and structure: $C_{10}H_{13}ClO$	Cosmetic biocides, denaturants, deodorant agents, oral care agents, and preservatives	Pepe et al. (2002)
			
Mixed Cresols	A mixture of <i>o</i> -, <i>m</i> -, and <i>p</i> -Cresol, each of which conforms to the formula: $C_7H_8O$ (see structures below)	Preservatives	Pepe et al. (2002)
<i>m</i> -Cresol	The phenol that conforms to the structure:	Preservatives, fragrance ingredients	Pepe et al. (2002)
			
<i>o</i> -Cresol	The phenol that conforms to the structure:	Preservatives, fragrance ingredients	Pepe et al. (2002)
			

(Continued on next page)

**TABLE 1**  
Ingredient definition and structure

Ingredient	Description	Uses	Reference
<i>p</i> -Cresol	<p>The phenol that conforms to the structure:</p> 	Preservatives, fragrance ingredients	Pepe et al. (2002)
Isopropyl Cresols	<p>The general mixture of isomeric methyl and propyl substituted phenols that conforms to the formula: <math>C_{10}H_{14}O</math></p> 	Preservatives, cosmetic biocides	Pepe et al. (2002)
Thymol	<p>The substituted phenol that conforms to the formula: <math>C_{10}H_{14}O</math></p> 	Denaturants, fragrance ingredients, vermifuge	Pepe et al. (2002); Grant (1972)
<i>o</i> -Cymen-5-ol	<p>The substituted phenol that conforms to the formula: <math>C_{10}H_{14}O</math></p> 	Cosmetic biocides	Pepe et al. (2002)
Carvacrol	<p>The substituted phenol that conforms to the formula: <math>C_{10}H_{14}O</math></p> 	Disinfectant, fungicide, fragrance ingredients, vermifuge	Infoplease Dictionary (2002); Woordenboek Organische Chemie (2000); Grant (1972)

**TABLE 2**

List of synonyms and trade names

Ingredient	Synonyms and trade names
Sodium <i>p</i> -Chloro- <i>m</i> -Cresol	sodium 3-methyl-4-chlorophenolate Preventol CMK sodium salt [Bayer AG] sodium <i>p</i> -chloro- <i>m</i> -cresolate
<i>p</i> -Chloro- <i>m</i> -Cresol	4-chloro- <i>m</i> -cresol 6-chloro- <i>m</i> -cresol 4-chloro-1-hydroxy-3-methylbenzene 2-chloro-hydroxytoluene 2-chloro-5-hydroxytoluene 4-chloro-3-hydroxytoluene 6-chloro-3-hydroxytoluene 2-chloro-5-methylphenol 4-chloro-3-methyl Phenol chlorocresol <i>p</i> -chlorocresol 3-hydroxy-4-chlorotoluene 3-methyl-4-chlorophenol parachlorometacresol PCMC
Chlorothymol	4-chloro-6-isopropyl-3-methylphenol 6-chlorothymol 4-chloro-5-methyl-2-(1-methylethyl)phenol
Mixed Cresols	cresol(s) (mixed) methylphenol ballicol tekeresol ar-toluenol tricresol cresylic acid
<i>m</i> -Cresol	3-methylphenol <i>m</i> -methylphenol 3-cresol <i>m</i> -hydroxytoluene <i>m</i> -toluol 3-hydroxytoluene bactinin gallex
<i>o</i> -Cresol	2-methylphenol 2-cresol <i>o</i> -cresylic acid <i>o</i> -hydroxytoluene <i>o</i> -toluol 2-hydroxytoluene

**TABLE 2**List of synonyms and trade names (*Continued*)

Ingredient	Synonyms and trade names
<i>p</i> -Cresol	4-methylphenol 4-cresol <i>p</i> -cresylic acid <i>p</i> -hydroxytoluene 4-hydroxytoluene <i>p</i> -toluol
Isopropyl Cresols	none found
Thymol	3-hydroxy- <i>p</i> -cymene <i>p</i> -cymen-3-ol 5-methyl-2-isopropyl-1-phenol 5-methyl-2-(1-methylethyl)phenol 2-hydroxy-1-isopropyl-4-methylbenzene isopropyl- <i>m</i> -Cresol
<i>o</i> -Cymen-5-ol	3-methyl-4-(1-methylethyl)phenol isopropyl methylphenol Biosol 3-methyl-4-isopropylphenol 2-isopropyl-5-hydroxytoluene <i>p</i> -thymol 4-isopropyl- <i>m</i> -cresol 4-isopropyl-3-methylphenol 5-oxy-1-methyl-2-isopropyl-benzol
Carvacrol	2-hydroxy- <i>p</i> -cymene isopropyl- <i>o</i> -Cresol

*References:* Budavari 1989; ChemIDplus 2002; CTFA 1981; Greenberg and Lester 1954; Hazardous Substances Database (HSDB) 2000; Lewis 1993, 1997, 2000; Kitazawa 1955; NLM 1983; Pepe et al. 2002; Registry of Toxic Effects of Chemical Substances (RTECS) 1993, 2000.

### Impurities

PCMC contains less than 0.1% 3-methylphenol (*m*-cresol) as measured by high-performance liquid chromatography (HPLC) and gas chromatography-mass spectrometry (GC-MS) (Andersen and Hamann 1984a).

Synthetic Thymol and Carvacrol were 98% pure from Aldrich Chemical Company (Milwaukee, WI). The impurities were not stated (Curtis et al. 1996).

According to the WHO (1995), commercial Cresols are manufactured in a wide range of grades and purities to suit the user's

**TABLE 3a**  
Physical and chemical properties of cresols

Property	<i>p</i> -Chloro- <i>m</i> -Cresol	Mixed Cresols	<i>m</i> -Cresol	<i>o</i> -Cresol	<i>p</i> -Cresol
Molecular weight	142.58 <sup>a</sup>	108.15 g <sup>b</sup> 108.13 g <sup>a</sup> 108.14 g <sup>i</sup>	108.15 g <sup>b</sup> 108.14 g <sup>a,i</sup> 108.1 <sup>r</sup>	108.15 g <sup>b</sup> 108.14 g <sup>a,i</sup> 108.1 <sup>r</sup>	108.15 g <sup>b</sup> 108.14 g <sup>a,i</sup> 108.1 <sup>r</sup>
Odor	Odorless when pure, usually Phenolic <sup>a</sup> ; phenolic <sup>z</sup>	Phenolic <sup>a</sup>	Phenolic <sup>a</sup>	Phenolic <sup>a</sup>	Phenolic <sup>a</sup>
Description	Colorless or white crystals or crystalline powder <sup>y</sup> Dimorphous crystals <sup>a</sup> White or slightly pink crystals <sup>z</sup>	Colorless, yellowish, brownish-yellow, or pinkish liquid that becomes darker with age and upon exposure to light <sup>d</sup>	Colorless or yellowish liquid <sup>a</sup>	Crystals or liquid that becomes darker with age and upon exposure to light <sup>d</sup>	Crystals <sup>a</sup>
Density	NA	1.030–1.047 <sup>b</sup>	1.034 <sup>a</sup>	1.047 <sup>a</sup>	1.0341 <sup>a</sup>
Octanol/water partition coefficient	3.00 <sup>h</sup> 3.10 <sup>h</sup> 3.14 <sup>h</sup>	No data <sup>g</sup>	1.96 <sup>g</sup> 2.00 <sup>r</sup>	1.95 <sup>g</sup> 2.00 <sup>r</sup>	1.94 <sup>g</sup> 1.93 <sup>r</sup>
Melting point	66°C <sup>aa</sup> 55.5°C and 66°C (ligroin) <sup>a</sup> 64–66°C <sup>z</sup> 235°C <sup>a,z,aa</sup>	10.9°–35.5°C <sup>b</sup>	11–12°C <sup>a</sup> 10.9°C <sup>b</sup> 12°C <sup>r</sup> 202°C <sup>a</sup>	30°C <sup>a</sup> 31°C <sup>r</sup> 191–192°C <sup>a</sup>	35.5°C <sup>a</sup> 35°C <sup>r</sup> 201.8°C <sup>a</sup>
Boiling Point		191°–203°C <sup>b</sup>			

Solubility	Somewhat soluble in water; very soluble in organic solvents <sup>a,a</sup> Solubility in water is 4.0 g/l <sup>b,b</sup> Freely soluble in alcohol, benzene, chloroform, ether, acetone, petroleum ether, fixed oils, terpenes, aqueous alkaline solutions; 1 g dissolves in 260 ml water (20°C), more soluble in hot water <sup>d</sup> Soluble in alkalies, organic solvents, fats, oils; soluble 1:250 in water at 25°C <sup>z</sup>	Soluble in alcohol, glycol, dilute alkalies, and water <sup>b</sup>	Soluble in about 40 parts of water, in solutions of fixed alkali hydroxides; miscible with alcohol, chloroform, ether <sup>d</sup>	Soluble in about 40 parts of water, in solutions of fixed alkali hydroxides; miscible with alcohol, chloroform, ether <sup>d</sup>	Soluble in aqueous solutions of alkali hydroxides; in the usual organic solvents <sup>d</sup>
Vapor pressure	—	0.975 (at 38–53°C)	0.143	0.31	0.13
UV max	—	—	271 nm <sup>q</sup> 278 nm <sup>p</sup>	270 nm <sup>o</sup> 276 nm <sup>p</sup> 271 nm <sup>q</sup> 10.17 <sup>k</sup>	275 nm <sup>l</sup> 284 nm <sup>p</sup> 271 nm <sup>q</sup> 10.26 <sup>e</sup> 10.17 <sup>k</sup>
pK <sub>a</sub> value	10.7 <sup>b,b</sup>	—	10.01 <sup>k</sup>	—	—
Specific rotation	—	+12° — +13° <sup>c</sup>	—	—	—
Stability	Volatile with steam <sup>a,z</sup> ; aqueous solution turns yellow on exposure to air or light <sup>a</sup>	—	—	—	—
Optimum pH	More active in acid than alkaline solutions <sup>a</sup>	—	—	—	—

See Table 3b for references.

**TABLE 3b**  
Physical and chemical properties of isopropyl cresols

Property	Isopropyl Cresols	Chlorothymol	Thymol	<i>o</i> -Cymen-5-ol	Carvacrol
Molecular weight	150.24 g <sup>b</sup>	184.66 g <sup>a</sup>	150.21 g <sup>a</sup> 150.24 g <sup>b</sup>	150.22 g <sup>d</sup>	150.21 g <sup>a</sup>
Odor	—	—	Characteristic <sup>a</sup> , thyme <sup>n</sup> Crystals <sup>a</sup> ; white crystalline solid <sup>d</sup>	odorless <sup>d</sup> Colorless or white powder, needlelike crystal <sup>d</sup>	Thymol odor <sup>a</sup> , mint <sup>n</sup> Liquid <sup>a</sup>
Density	—	—	0.9699 <sup>a</sup>	—	0.976 <sup>a</sup>
Octanol/water partition coefficient	—	4.344 <sup>s</sup> 3.92 <sup>t</sup>	3.30 <sup>h</sup> 3.376 <sup>k</sup>	—	3.376 <sup>k</sup>
Melting point	—	59°–61°C <sup>u</sup> 62°–64°C <sup>a</sup> 62°C <sup>s</sup>	51.5°C <sup>a</sup> , 52°C <sup>d</sup> 51°C <sup>k</sup>	110°–113°C <sup>d</sup> 111°–112°C <sup>v,w</sup>	~ 0°C <sup>a</sup>
Boiling point	—	NA	~233°C <sup>a</sup> , 232°C <sup>j</sup>	244°C <sup>f</sup> , 238°C <sup>j</sup>	237°–238°C <sup>a</sup>
Solubility	—	1 mg dissolves in 1000 ml of water, 2 ml benzene, 2 ml chloroform, 1.5 ml ether, and 10 ml petroleum ether <sup>a</sup>	Soluble in glacial acetic acids, oils, fixed alkali hydroxides <sup>a</sup>	Sol (26°–28°C) in water (0.03–0.04%), ethanol (36%), isopropanol (50%), ethylene glycol (3.5%), propylene glycol (8%), glycerin (0.1%) <sup>d</sup> ~ 272 nm <sup>d</sup> ~ 275 nm <sup>v,w</sup>	Soluble in alcohol or ether and practically insoluble in water <sup>a</sup>
UV max	—	—	—	—	NA
pK <sub>a</sub> Value	—	—	10.35 <sup>k</sup>	—	10.40 <sup>k</sup>
pH	—	—	—	Neutral <sup>v,w</sup>	3.9
Hansch $\pi$ parameter	—	—	—	1.58 <sup>x</sup>	NA

*References:* <sup>a</sup>Budavari (1989); <sup>b</sup>Lewis (1997); <sup>c</sup>United States Pharmacopeial Conventions (2000); <sup>d</sup>Kabara (1984); <sup>e</sup>Lide (1993); <sup>f</sup>Nikitakis and McEwen (1990); <sup>g</sup>International Programme on Chemical Safety (1995); <sup>h</sup>Leo et al. (1971); <sup>i</sup>American Conference of Governmental Hygienists (ACGIH) (2000); <sup>j</sup>National Academy of Sciences (1996); <sup>k</sup>Barratt (1996); <sup>l</sup>Bossert and Young (1986); <sup>m</sup>Barratt et al. (1998); <sup>n</sup>He et al. (1997); <sup>o</sup>Masunaga et al. (1986); <sup>p</sup>Trost et al. (1995); <sup>q</sup>Bund et al. (1995); <sup>r</sup>Fiserova-Bergerova et al. (1990); <sup>s</sup>Ravanel et al. (1985); <sup>t</sup>Pišelová et al. (1996); <sup>u</sup>Nikitakis and McEwen (1990); <sup>v</sup>Kitazawa (1955); <sup>w</sup>CTFA (1981); <sup>x</sup>Kuchar et al. (1974); <sup>y</sup>Yakuji Nippo, Ltd. (1985); <sup>z</sup>Lewis (1993); <sup>aa</sup>Sax (1979); <sup>bb</sup>Paulus and Genth (1983); <sup>cc</sup>WHO (1995);

**TABLE 4**  
Analytical methods for Cresols and Isopropyl Cresols

Ingredient	Analytical method	Matrix (if given)	Reference
PCMC	High-performance liquid chromatography and gas chromatography-mass spectrometry	—	Andersen and Hamann (1984a)
	A saturated solution of PCMC can be identified by adding one drop of ferric chloride test solution to the solution and shaking; a light blue-purple color develops	—	Yakuji Nippo Ltd. (1985)
<i>m</i> -Cresol	Gas chromatography with flame ionization detection	Urine from human exposure to toluene or benzene vapors	Balikova and Kohlicek (1989)
	Gas chromatography-mass spectroscopy	Sample of Asuka carbonaceous chondrites	Naraoka et al. (1999)
	Gas chromatography-mass spectroscopy	Urine from humans exposed to phenol, Cresols, xylenols, and other phenolic derivatives	Bieniek (1997)
	Gas chromatography-mass spectroscopy	Blood from humans	Hart and Dasgupta (1997)
	Gas chromatography-mass spectroscopy	Specific effluent of petrochemical refinery	Charest et al. (1999)
	Gas chromatographic determination after acid hydrolysis/extractive acetylation	Urine after human exposed to toluene	Weber (1992)
	Gas chromatography with electron capture detection	Human urine	Dills et al. (1997); Pierce et al. (1998)
	Reversed-phase liquid chromatographic column-switching system	From groundwater microcosm in the presence of toluene	Chamkasen et al. (1991)
	Wide-bore capillary gas chromatography with flame ionization detection and nitrogen phosphorous detectors	Human blood and urine	Kageura et al. (1989)
	Kinetic wavelength-pair method	Human urine	Carreto et al. (1996)
	High-performance liquid chromatography	Human urine	Brega et al. (1990); Moon et al. (1997)
	Thin layer chromatography with semiquantitative visual determination method	Waste water of the petroleum industry	Liu et al. (1987a)
	Comparison of its infrared spectrum, nuclear magnetic resonance, and mass spectral data	Urine from rats dosed orally with <i>R</i> -(+)-pulegone	Madyastha and Raj (1993)
<i>o</i> -Cresol	Gas chromatography with flame ionization detection	Urine after humans exposure to toluene or benzene vapors	Balikova and Kohlicek (1989); Amorim and Alvarez-Leite (1996); Tardif et al. (1998)
	Gas chromatographic determination after acid hydrolysis/extractive acetylation	Urine after human exposure to toluene	Weber (1992)

(Continued on next page)

**TABLE 4**Analytical methods for Cresols and Isopropyl Cresols (*Continued.*)

Ingredient	Analytical method	Matrix (if given)	Reference
	Gas chromatography–mass spectroscopy	Sample of Asuka carbonaceous chondrites	Naraoka et al. (1999)
	Gas chromatography–mass spectroscopy	Urine from humans exposed to phenol, Cresols, xylenols, and other phenolic derivatives	Bieniek (1997)
	Gas chromatography–mass spectroscopy	Specific effluent of petrochemical refinery	Charest et al. (1999)
	Gas chromatography–mass spectroscopy	Sample of shale oil	May and Chesler (1980)
	Gas chromatography with electron capture detection	Human urine	Dills et al. (1997); Pierce et al. (1998)
	High-performance liquid chromatography	Urine from humans after exposure to toluene	Baelum et al. (1993)
	High-performance liquid chromatography	Human urine	Brega et al. (1990); Moon et al. (1997)
	High-performance liquid chromatography	Urine and blood of male cats exposed to tri-o-cresyl phosphate	Nomeir and Abou-Donia (1983); Nomeir and Abou-Donia (1986)
	High-performance liquid chromatography	Amount in a medium inoculated with the fungus <i>Rhizoctonia praticola</i>	Bollag and Shuttleworth (1988)
	Thin layer chromatography	Urine from humans exposed to phenol, Cresols, xylenols, and other phenolic derivatives	Bieniek (1994)
	Thin layer chromatography with semi-quantitative visual determination method	Waste water of the petroleum industry	Liu et al. (1987a)
<i>p</i> -Cresol	Gas chromatography with flame ionization detection	Urine after human exposure to toluene or benzene vapors	Balikova and Kohlicek (1989)
	Gas chromatography–mass spectroscopy	Sample of Asuka carbonaceous chondrites	Naraoka et al. (1999)
	Gas chromatography–mass spectroscopy	Urine from humans exposed to phenol, Cresols, xylenols, and other phenolic derivatives	Bieniek (1997)
	Gas chromatography–mass spectroscopy	Specific effluent of petrochemical refinery	Charest et al. (1999)
	Gas chromatography–mass spectroscopy	From a water sample	Onodera et al. (1986)
	Gas chromatographic determination after acid hydrolysis/extractive acetylation	Urine after human exposure to toluene	Weber (1992)
	Gas chromatography with electron capture detection	Human urine	Dills et al. (1997); Pierce et al. (1998)
	Wide-bore capillary gas chromatography with flame ionization detection and nitrogen phosphorous detectors	Human blood and urine	Kageura et al. (1989)
	Kinetic wavelength-pair method	Human urine	Carreto et al. (1996)
	High-performance liquid chromatography	Human urine	Brega et al. (1990); Moon et al. (1997)
			(Continued on next page)

**TABLE 4**  
Analytical methods for Cresols and Isopropyl Cresols (*Continued*)

Ingredient	Analytical method	Matrix (if given)	Reference
	High-performance liquid chromatography	Human urine and feces	Birkett et al. (1995)
	High-performance liquid chromatography	Amount in a medium inoculated with the fungus <i>Rhizoctonia praticola</i>	Bollag and Shuttleworth (1988)
	High-performance liquid chromatography	Fluorescence detector quantified serum <i>p</i> -Cresol in uremic patients on hemodialysis	Niwa (1993)
	Thin layer chromatography and column chromatography over silica (or alumina) gel	Urine of rats treated with menthofuran (or piperitenone)	Madyastha and Raj (1991); Madyastha and Gaikwad (1999)
	Thin layer chromatography	Urine from humans exposed to phenol, Cresols, xlenols, and other phenolic derivatives	Bieniek (1994)
	Thin layer chromatography with semi-quantitative visual determination method	Waste water of the petroleum industry	Liu et al. (1987a)
	Mass spectrometry using a silicone membrane source	Hydrolysed urine from workers exposed to toluene	Sturaro et al. (1989)
<i>o</i> -Cymen-5-ol	High-performance liquid chromatographic analysis to determine <i>o</i> -Cymen-5-ol at concentrations of 2.5–12.5 ppm	—	Ohnishi et al. (1980); Kazama et al. (1980)
	Use of gas chromatography has also been reported	—	Leston (1975)
Thymol	Capillary gas chromatography–mass spectroscopy	Urine of male albino rats	Austgulen et al. (1987)
	Gas chromatography and thin-layer chromatography	Urine of rabbits and humans	Takada et al. (1979)
	Gas chromatography with flame ionization detection	Essential oils of aromatic plants growing wild in Turkey	Muller-Riebau et al. (1995)
Carvacrol	Gas chromatography with flame ionization detection	Essential oils of aromatic plants growing wild in Turkey	Martini et al. (1996); Muller-Riebau et al. (1995)
	Capillary gas chromatography–mass spectroscopy	Urine of male albino rats	Austgulen et al. (1987)

requirements. Technical grade Cresol is typically 40% *m*-Cresol, 30% *p*-Cresol, and 20% *o*-Cresol, and 10% phenol. The individual isomers are available at purity amounts from 85% to >99% from chemical suppliers in the USA.

### Ultraviolet Absorbance

*m*-Cresol absorbs in the UVB range, with maximum absorbance no greater than 300 nm (Dupont et al. 1990). A study by Etzkorn et al. (1999) reported that *m*-Cresol, *o*-Cresol, and *p*-Cresol absorbed in the UVB range, with maximum absorbances at 277.9, 275.8, and 283.0 nm, respectively.

### USE

#### Cosmetic

As given in the *International Cosmetic Ingredient Dictionary and Handbook*, Sodium *p*-Chloro-*m*-Cresol is an organic compound that functions as a preservative and PCMC functions as a cosmetic biocide and as a preservative in cosmetic formulations (Pepe et al. 2002).

Mixed Cresols is a mixture of *o*-Cresol, *m*-Cresol, and *p*-Cresol which functions as a preservative (Pepe et al. 2002).

*m*-Cresol and *o*-Cresol function as both a preservative and fragrance ingredient (Pepe et al. 2002).

*p*-Cresol functions as both preservatives and fragrance ingredients (Pepe et al. 2002). In a preliminary study, Rastogi et al. (1998) analyzed 71 deodorants for identification of nonfragrance and fragrance materials present and the sensitizing potential of these ingredients. A mixture of *o*-Cresol and *p*-Cresol was found in two vapo-spray and one aerosol spray deodorants but neither isomer had a chemically reactive site present in the structure that is associated with sensitizing potential.

Isopropyl Cresols is the general mixture of isomeric methyl and propyl substituted phenols that functions as preservatives and cosmetic biocides in cosmetic formulations (Pepe et al. 2002).

Thymol functions as a denaturant and fragrance ingredient in cosmetic formulations (Pepe et al. 2002; Grant 1972).

*o*-Cymen-5-ol functions as a cosmetic biocide in cosmetic formulations (Pepe et al. 2002).

As noted earlier, Carvacrol not listed in the *International Cosmetic Ingredient Dictionary and Handbook*, but Grant (1972) and the Infoplease Dictionary (2002) stated that it is primarily used as a disinfectant, fungicide, and fragrance ingredient in cosmetic formulations.

Data submitted to CIR from the Food and Drug Administration (FDA) in 2001 reported no uses of Sodium *p*-Chloro-*m*-Cresol, Chlorothymol, Mixed Cresols, *m*-Cresol, *o*-Cresol, *p*-Cresol, Isopropyl Cresols, or Carvacrol. FDA did have reported use data in 2001 for *p*-Chloro-*m*-Cresol, Thymol, and *o*-Cymen-5-ol and these data are given in Table 5. The total number of a given product type (e.g. 881 moisturizing creams) reported to FDA may be used to gain perspective on how widely a particular ingredient is used. For example, 2 of 881 moisturizing creams reportedly contained *p*-Chloro-*m*-Cresol.

Concentration of use values are no longer reported to the FDA by the cosmetic industry (FDA 1992), but a survey conducted by CTFA found current concentrations of use for Thymol and *o*-Cymen-5-ol. These data are included in Table 5. Historical concentrations of use for Thymol were in the 0.1% to 1% and the 1% to 5% range; and for *o*-Cymen-5-ol were in the 0% to 0.1% range (FDA 1984).

The Japanese Ministry of Health, Labor, and Welfare (MHLW) has restricted PCMC, when used as a preservative, to 0.50 g per 100 g in all cosmetics (MHLW 2000a). In Japan, PCMC is considered a quasidrug, requiring that it is labeled appropriately in products used directly on the body.

The MHLW also has restricted Cresol, when used as a preservative, to 0.010 g per 100 g of rinse-off or leave-on products; and Cresol is not permitted for use in cosmetics that are applied on mucous membranes. In Japan, Cresol is considered a quasidrug, which is defined as having “a mild effect on the body, but are neither intended for the diagnosis, prevention, or treatment of disease, nor to affect the structure or function of the body.” Cresol’s status as a quasidrug requires that it be labeled appropriately in products used both directly on the body and those that cannot be used directly on the body (MHLW 2000a).

The Japanese MHLW have placed restrictions on Thymol when used as a preservative. Thymol as a preservative is restricted to 0.05 g per 100 g of rinse-off or leave-on products and is permitted for use in cosmetics used on mucous membranes, when limited to the mouth (MHLW 2000a). In Japan, Thymol is considered a quasidrug and must be labeled appropriately in products used directly on the body (MHLW 2000b).

PCMC is approved for use by members of the European Union as a preservative at a maximum authorized concentration of 0.2% and is prohibited for use in products intended to come in contact with mucous membranes; PCMC “may also be added to cosmetic products in concentration other than those laid down in this Annex [Annex VI; Dir.76/768/EEC—June 1986] for other specific purposes apparent from the presentation of the products, e.g., as deodorants in soaps or as anti-dandruff agents in shampoos” (EEC Cosmetics Directive 1993).

According to Cronin (1980), cosmetic chemists may avoid using PCMC in their formulations as it may interfere with perfumes.

## Noncosmetic

### *Foods and Drugs*

**PCMC.** PCMC has applications as a topical antiseptic, disinfectant, and preservative in pharmaceutical products (Kabara 1984). Because of its antiseptic properties, it is commonly used in heparin solutions, in electrode paste, and in various creams used in dermatology and general skin care (Dossing et al. 1986). It is an efficient bactericide that is more effective in acid than in alkaline solutions (Cronin 1980). PCMC can also be used in veterinary topical medicaments and in veterinary intrauterine lubricants as an antiseptic and preservative at 0.1% to 0.2% (Rossoff 1974).

PCMC is approved for use as an indirect food additive (Rothschild 1990). Nonfood or medical uses of PCMC include packaging materials, adhesives, glues, inks, paints and varnishes, textile finishes, leather and tanning agents, and industrial oils and emulsions (Dooms-Goossens et al. 1981).

The use concentration for most noncosmetic applications of PCMC ranges from 0.05% to 0.5% (Andersen and Hamann 1984b); aqueous drugs for parenteral use are sometimes preserved with 0.05% to 0.1% PCMC (Cronin 1980).

**Mixed Cresols.** As determined by FDA and codified in the Code of Federal Regulations (CFR), there are inadequate data to establish general recognition of the safety and effectiveness of Mixed Cresols in some over-the-counter (OTC) drug products. Mixed Cresols has not been established as safe and effective in the following drug products: dandruff/seborrheic dermatitis/psoriasis, oral health care products (antimicrobial), and astringent products (21CFR310.545).

***m*-Cresol and *o*-Cresol.** *m*-Cresol and/or *o*-Cresol can be reacted with formaldehyde to form phenolic resins as the basic polymer for use in polymeric coatings that may be safely used as the food-contact surface of articles intended for use in producing,

## COSMETIC INGREDIENT REVIEW

**TABLE 5**Product formulation and concentration of use data on *p*-Chloro-*m*-Cresol, thymol, and *o*-Cymen-5-ol

Product category (number of formulations reported to FDA in 2001) (FDA 2001)	Number of formulations containing ingredient (FDA 2001)	Maximum current concentration of use (CTFA 2001)
<i>p</i> -Chloro- <i>m</i> -Cresol		
Skin care preparations		
Moisturizers (881)	2	—
<i>p</i> -Chloro- <i>m</i> -Cresol totals	2	—
Thymol		
Noncoloring hair preparations		
Hair conditioners (630)	1	—
Shampoos (851)	—	—
Hair tonics, dressings, etc. (577)	—	0.004%
Hair coloring preparations		
Hair dyes and colors (1588)	1	—
Hair tints (49)	1	—
Makeup preparations		
Lipstick (942)	—	—
Other (186)	—	—
Nail care products		
Other (57)	—	0.01%
Oral hygiene products		
Dentifrices (38)	—	0.25%
Mouthwashes and breath fresheners (46)	4	—
Personal hygiene products		
Douches (5)	2	—
Skin care preparations		
Skin cleansing creams, lotions, etc. (733)	—	—
Foot powders and sprays (35)	1	—
Paste masks (mud packs) (269)	1	—
Skin fresheners (181)	—	—
Other (715)	—	0.06%
Thymol totals	11	0.004–0.25%
<i>o</i> -Cymen-5-ol		
Eye makeup		
Eyeshadow (551)	—	—
Mascara (187)	2	—
Other eye makeup preparations (151)	—	—
Fragrance preparations		
Perfumes (227)	—	0.5%
Hair coloring preparations		
Hair conditioners (630)	—	0.1%
Makeup preparations		
Foundations (319)	—	—
Makeup bases (136)	1	—
Nail care products		
Nail creams and lotions (15)	—	—
Shaving preparations		
Other (61)	1	—
Skin care preparations		
Skin cleansing creams, lotions, etc. (733)	—	—
Face and neck skin care (304)	—	—
Body and hand skin care (827)	3	—
Moisturizers (881)	1	—
Night skin care (200)	—	—
Paste Masks (mud packs) (269)	1	—
Suntan preparations		
Other (37)	—	—
<i>o</i> -Cymen-5-ol totals	9	0.1–0.5%

manufacturing, packing, processing, preparing, treating, packaging, transporting, or holding food (21CFR175.300).

*m*-Cresol and/or *o*-Cresol can be used to produce phenolic resins and used safely as the food-contact surface of molded articles intended for repeated use in contact with nonacid food (pH above 5.0). The phenolic resins are produced from one or more selected phenols and one or more selected aldehydes (phenols: *p*-tert-amylphenol, *p*-tert-butylphenol, *m*-, *o*-, and *p*-Cresol, *p*-octylphenol, phenol, and *o*- and *p*-phenylethylphenol mixture produced when phenol is made to react with styrene in the presence of sulfuric acid catalyst; aldehydes: acetaldehyde, formaldehyde, and paraldehyde). The extracted phenol should not exceed 0.005 mg per square inch of food-contact surface and, in accordance with good manufacturing practice, finished molded articles containing phenolic resins shall be thoroughly cleansed prior to first use in contact with food (21CFR177.2410).

Hamaguchi and Tsutsui (2000) noted that *m*-Cresol is used as a topical dental antiseptic, and Wappler et al. (1996) reported that *m*-Cresol is used in insulin preparations, and is suspected to be a trigger of malignant hyperthermia. FDA determined that *m*-Cresol has not been established as safe and effective in topical antifungal drug products (21CFR310.545).

*p*-Cresol. *p*-Cresol can be reacted with formaldehyde to form phenolic resins as the basic polymer for use in polymeric coatings that may be safely used as the food-contact surface of articles intended for use in producing, manufacturing, packing, processing, preparing, treating, packaging, transporting, or holding food (21CFR175.300).

*p*-Cresol can be used to produce phenolic resins and used safely as the food-contact surface of molded articles intended for repeated use in contact with nonacid food (pH above 5.0). The phenolic resins are produced from one or more selected phenols and one or more selected aldehydes (phenols: *p*-tert-amylphenol, *p*-tert-butylphenol, *m*-, *o*-, and *p*-Cresol, *p*-octylphenol, phenol, and *o*- and *p*-phenylethylphenol mixture produced when phenol is made to react with styrene in the presence of sulfuric acid catalyst; aldehydes: acetaldehyde, formaldehyde, and paraldehyde). The extracted phenol is not to exceed 0.005 mg per square inch of food-contact surface and in accordance with good manufacturing practice, finished molded articles containing phenolic resins shall be thoroughly cleansed prior to first use in contact with food (21CFR177.2410).

*p*-Cresol can be used as a synthetic flavoring and adjuvant in food at the minimum quantity required to produce the intended effect and in accordance with good manufacturing practices (21CFR172.515).

*Isopropyl Cresols*. Isopropyl Cresol may be safely used as components of articles used in packaging, transporting, or holding of food so that exposure to Isopropyl Cresol is minimized (21CFR175.105) and can be used as a component of the uncoated or coated food-contact surface of paper and paperboard intended for use in producing, manufacturing, packing, processing, preparing, treating, packaging, transporting, or holding dry food as used in amounts not to exceed that required quantity to

produce the intended physical or technical effect and in accordance with good manufacturing practices (21CFR172.180).

Isopropyl Cresol is approved for use only as an antioxidant for fatty based coating adjuvant provided it is used at a level not to exceed 0.005% by weight of coating solids (21CFR176.170).

*Chlorothymol*. There are inadequate data to establish general recognition of the safety and effectiveness of Chlorothymol in some OTC drug products. Chlorothymol has not been established as safe and effective in the following drug products: diaper rash drug products (21CFR310.545).

Chlorothymol is a denaturant authorized for use in specially denatured alcohol (27CFR21.151). Ten pounds of Chlorothymol (or two or more oils and specific substances) can be added to every 100 gallons of alcohol and its use as a solvent is authorized in hair and scalp preparations, bay rum, lotions and creams (hand, face, and body), body deodorants, perfumes and perfume tinctures, toilet waters and cologne, dentifrices, mouthwashes, shampoos, soap and bath preparations, external pharmaceutical (not United States Pharmacopeia [USP] or National Formulary [NF]), liniments (USP or NF), antiseptic solutions (USP or NF), miscellaneous external pharmaceuticals (USP or NF), miscellaneous drug processing (including pill manufacture), disinfectants, insecticides, fungicides, and other biocides, sterilizing and preserving solutions, and theater sprays, incense, and room deodorants (27CFR21.65).

*Thymol*. Thymol can be used as a synthetic flavoring and adjuvant in food at the minimum quantity required to produce the intended effect and in accordance with good manufacturing practices (21CFR172.515).

There are inadequate data to establish general recognition of the safety and effectiveness of Thymol in some OTC drug products. Thymol has not been established as safe and effective in the following drug products: topical acne products, nasal decongestant products, dandruff/seborrheic dermatitis/psoriasis products, external analgesic and anesthetic products, fever blister and cold sore treatment products, poison ivy, poison oak, and poison sumac products, oral health care products (nonantimicrobial), astringent products, and topical antifungal products (21CFR310.545).

Ettayebi et al. (2000) reported that Thymol in conjunction with nisin can be used for effective control of food-borne pathogenic bacteria. FDA has determined that Thymol may be safely used as components of articles used in packaging, transporting, or holding of food so that exposure to Thymol is minimized. The use of Thymol is restricted to that of a preservative (21CFR175.105).

Thymol is a denaturant authorized for use in specially denatured alcohol (27CFR21.151). Ten pounds of Thymol (or two or more oils and specific substances) can be added to every 100 gallons of alcohol and its use as a solvent is authorized in hair and scalp preparations, bay rum, lotions and creams (hand, face, and body), body deodorants, perfumes and perfume tinctures, toilet waters and cologne, dentifrices, mouthwashes, shampoos, soap and bath preparations, external pharmaceutical (not USP or

NF), liniments (USP or NF), antiseptic solutions (USP or NF), miscellaneous external pharmaceuticals (USP or NF), miscellaneous drug processing (including pill manufacture), disinfectants, insecticides, fungicides, and other biocides, sterilizing and preserving solutions, and theater sprays, incense, and room deodorants (21CFR21.65).

Thymol is generally recognized as safe (GRAS) in animal feed as a source of trace minerals (21CFR582.80).

Thymol is used topically for treatment of acne vulgaris, hemorrhoids, and local analgesia, as well as in cough drops, vaginal douches, and toothpastes (Lorenzi et al. 1995).

Thymol is an ingredient used in Vicks throat lozenge. Thymol is considered safe but does not have proven efficacy as an anesthetic for topical use on the throat and its use has decreased in recent times (Gossel 1985).

*o-Cymen-5-ol.* *o*-Cymen-5-ol is commonly sold for use as an antimold and antimicrobial agent, as a preservative, and as an antioxidant (CTFA 1981).

*o*-Cymen-5-ol is used in a number of OTC drugs in Japan (Osaka Kasei Co., Ltd. 1992), including four urogenital and anal drug products at a maximum amount of  $\leq 0.5\%$  and  $\leq 2$  mg in ointments and suppositories, respectively; eight products for dermatological use for bactericides and disinfectants at a maximum concentration of  $\leq 3.0\%$  and  $\leq 0.03\%$  in ointments and solutions, respectively; two products for dermatological use for chilblains and chapped skin at a maximum concentration of 0.1% in ointments and creams; two products for dermatological use for pyogenic diseases at a maximum concentration of 0.3% and 0.05% in creams and ointments, respectively; 38 products for dermatological use for analgesics, antipruritics, astringents, antiphlogistics at a maximum concentration of  $\leq 1.5\%$  in creams,  $\leq 3.0\%$  in ointments,  $\leq 0.1\%$  in lotions,  $\leq 1.0\%$  in patches, and 0.1% in jellies and liniments; 10 products for dermatological use for parasitic dermal disease at a maximum concentration of  $\leq 3.0\%$  and  $\leq 1.0\%$  in ointments and solutions, respectively; and one oral health product at a maximum concentration of 0.05% in paste.

*Carvacrol.* Carvacrol can be used as a synthetic flavoring and adjuvant in food at the minimum quantity required to produce the intended effect and in accordance with good manufacturing practices (21CFR172.515). The Research Institute for Fragrance Materials (RIFM) specifically noted that Carvacrol is a flavor ingredient that can be found in alcoholic beverages, baked goods, chewing gum, condiment relish, frozen dairy, gelatin pudding, non-alcoholic beverages, and soft candy at concentrations from 0.1 to 28.54 ppm (RIFM 2001a).

#### Other Noncosmetic

*PCMC.* PCMC may be useful as a supplementary test for the diagnosis of malignant hyperthermia susceptibility (Ørding et al. 1997).

*Mixed Cresols.* Cresols are used as wire enamel solvents and as organic intermediates in phenolic resins and phosphate esters manufacturing. Cresols and their isomers are used in the

production of herbicides, as cleaning compounds, disinfectants, solvents, ore floatation, cleaners, as additives in motor oil, and as intermediates in production of phenolic resins and phosphate esters (Cheng and Kligerman 1984; Environmental Protection Agency 1983).

Cresols are used in the preparation of antiseptics and hydroquinone, have minor use as food preservatives, and as photographic chemicals (Dean 1978).

Cresols are used in disinfectants (Hart and Dasgupta 1997).

Cresols are readily biodegradable by various microorganisms and can be byproducts of industrial processes (Koppers Company, Inc. 1979).

*m-Cresol.* *m*-Cresol is one of the more abundant phenols in waste waters from hydrocarbon processing and coal conversion processes such as coking, gasification, and liquefaction (Fedorak and Hrudey 1986). *m*-Cresol is also found at sites contaminated by creosote (a wood preservative) (Goerlitz et al. 1985). *m*-Cresol can be metabolized by sulfate-reducing bacterial enrichments obtained from anoxic aquifers (Sulfita et al. 1989). *m*-Cresol is an important component of environmental tobacco smoke (ETS) (Daisey et al. 1998).

*o-Cresol.* *o*-Cresol is one of several toxic ingredients found in the smoke of a burning mosquito coil (Liu et al. 1987b), is an important component of ETS (Daisey et al. 1998), and is found in waste waters from the coal conversion processes (Neufeld 1984).

*o*-Cresol can be metabolized by sulfate-reducing bacterial enrichments obtained from anoxic aquifers (Sulfita et al. 1989).

*p-Cresol.* *p*-Cresol is an important component of ETS (Daisey et al. 1998), is found in waste waters from the coal conversion processes (Neufeld 1984), and is found naturally in ylang-ylang, jasmine, raspberry, cheese, coffee, and cocoa (Sinki and Schlegel 1990).

*p*-Cresol can be metabolized by sulfate-reducing bacterial enrichments obtained from anoxic aquifers (Sulfita et al. 1989).

*Thymol.* Thymol is an effective control on mosquito populations by reducing the egg hatchability and increasing the sterility in mosquitoes (Mansour et al. 2000).

Thymol is used as a reagent in the thymol turbidity test for globulin (Takada et al. 1979).

According to Grant (1972), Thymol functions as a vermicide.

*o-Cymen-5-ol.* An insect-repellent composition containing *o*-Cymen-5-ol as a component has been described by Inazuka and Tsuchiya (1979).

A number of patents that describe the use of *o*-Cymen-5-ol in the preparation of other phenolic compounds and in the stabilization of resin blends (Kato and Yatsu 1967; Perrin 1971; Biller and Kuehne 1973; Klein and Wedemeyer 1974; Kodama et al. 1975; Leston 1975) have also been issued.

*Carvacrol.* Grant (1972) reported that Carvacrol is used as a vermifuge. According to Ahn et al. (1995), Carvacrol was tested for its ability to repel mice from gnawing wire. It was suggested that Carvacrol could be a new preventive agent against various kinds of rodent damage.

Mansour et al. (2000) reported that Carvacrol is an effective control on mosquito populations by reducing the egg hatchability and inducing the sterility in mosquitoes.

## GENERAL BIOLOGY

### Absorption, Distribution, Metabolism, Excretion

**General.** The Research Triangle Institute (1992c) stated that Cresols can be absorbed through the skin, respiratory tract, and digestive tract. Cresols can penetrate deeply into tissues that are exposed. After Cresols are absorbed, most of the chemical is metabolized by the liver and either the metabolites or the unchanged chemical are excreted by the kidney with trace amounts excreted via the lungs. In vivo, the Cresol isomers are conjugated and excreted as glucuronides and sulfates. Significant amounts of Cresols are secreted in the bile and undergo enterohepatic recirculation. The kidney is the main route for removing Cresols.

*m*-Cresol, *o*-Cresol, and *p*-Cresol are biomarkers for phenol exposure. Bieniek (1994) assayed *o*-Cresol (found only in test subjects) and *p*-Cresol from human urine by thin-layer chromatograph (TLC). *o*-Cresol (76.9 mg/L) and *p*-Cresol (58.6 mg/L) were detected in the urine of workers employed in the distillation of the high temperature phenolic fraction of tar (carbolic oil) and in nonexposed workers at 68.1 g/L (*o*-Cresol) and 25.7 mg/L (*p*-Cresol). Bieniek (1997) assayed *m*-Cresol, *o*-Cresol and *p*-Cresol from human urine by GC-MS. All subjects were male smokers exposed mainly to phenol, Cresols, xlenols, and other phenolic derivatives while employed in the distillation of carbolic oil.

*o*-Cresol, *m*-Cresol, and *p*-Cresol are metabolites of toluene exposure and urinary levels are often used to monitor exposure to and metabolism of toluene. Detoxication of the Cresol isomers can last at least 24 h (Woiwode and Drysch 1981; Bælum et al. 1993; Tardif et al. 1998; and Truchon et al. 1999).

Hanioka et al. (1995) reported that *o*-Cresol and *p*-Cresol are minor metabolites of toluene in dog liver microsomes in vitro. Nakajima et al. (1997) reported that *o*-Cresol and *p*-Cresol are minor metabolites of toluene in human liver microsomes and formation of *o*-Cresol is elevated in the liver microsomes of smokers.

**PCMC.** Paulus and Genth (1983) reported a pharmacokinetic study in which rats were dosed orally with 300 mg/kg PCMC. PCMC reportedly was eliminated rapidly through the kidneys. There is no likelihood of cumulative effects. A corresponding examination of fatty and hepatic tissues from rats that were fed 150 to 1500 ppm PCMC for up to 13 weeks revealed no indication of an accumulation of PCMC in these tissues.

Four groups of conventional female albino guinea pigs, three per group, were used to determine the bioavailability of PCMC (Andersen et al. 1985). Occlusive patches of 0.2 ml of a 5% PCMC aqueous suspension stabilized with Carbomer 941, a saturated aqueous solution of 0.38% PCMC, 5% PCMC in olive oil/acetone (4/1), or 5% PCMC in propylene glycol were ap-

plied for 24 h. After 96 h, the animals were killed and the skin at the site of patch testing was removed for analysis (the patches were kept for analysis to determine the amount of PCMC remaining in the patch material). Fractional sampling of the urine and feces was performed to determine the rate of absorption of PCMC.

An additional three animals were injected with PCMC intraperitoneally to determine the excretion rate. However, no free PCMC was found, indicating rapid metabolism. The authors stated that further analysis would be required before PCMC absorption can be estimated by this method ( $[^{14}\text{C}]$ PCMC was not available).

In determining bioavailability, the authors based their calculations on the assumption that the saturated PCMC solution is 0.4% (w/v), corresponding to 0.8 mg in 0.2 ml, and that 0.2 ml of the 5% PCMC preparations contained 10 mg of the chemical. The results indicated that 25% of the aqueous PCMC (stabilized with carbomer 941) and 46% of the saturated aqueous PCMC solution remained in the patches. Only 0.2% of the aqueous PCMC (stabilized with carbomer 941) and 0.5% of the saturated aqueous PCMC solution was found in the skin at the patch site. This was compared to 65% of the PCMC in propylene glycol and 66% of the PCMC in olive oil/acetone solutions remaining in the patch; and 0.7% and 1.6%, respectively, found in the skin at the patch site.

The authors concluded that PCMC was more bioavailable from the aqueous preparations. After 96 h, 0.2% and 0.5% PCMC was detected at the patch test site in the animals dosed with 5% and saturated aqueous PCMC, respectively, and 0.7% and 1.6% PCMC were found in the skin of the animals patch tested with 5% PCMC in olive oil/acetone and propylene glycol, respectively (Andersen et al. 1985).

Huq et al. (1986) reported an in vitro study in which abdominal skin from SKH-hr-1 mice was used to determine the permeability of PCMC. The permeability was evaluated, with whole skin and skin that was stripped repeatedly (20 times) with cellophane tape to remove the stratum corneum, by mounting the excised samples in a two-compartment diffusion cell, with the two half-cells being filled with normal saline. Samples were withdrawn and assayed spectrophotometrically; the absorbance was measured at 245 nm. Permeability coefficients were assessed for both stripped and whole skin under conditions that kept ionization to a minimum.

In assessing the permeability of PCMC through whole skin, the concentration used was 0.05 g/100 ml, the  $\text{pK}_a$  of PCMC was 9.56, the donor pH was 6.18, and the receiver pH was 6.2. Under these conditions, the apparent permeability coefficient for whole skin was  $119 \pm 1.8 \times 10^{-3}$  cm/h and the average lag time was 35.8 min. For stripped skin, 0.055 g/100 ml PCMC was used and the donor and receiver pH was 6.2. The permeability coefficient of stripped skin was  $241 \pm 22 \times 10^{-3}$  cm/h and the lag time was 14.6 min. The estimated permeability coefficients of PCMC for viable tissue and stratum corneum were  $302 \times 10^{-3}$  and  $235 \times 10^{-3}$  cm/h, respectively (Huq et al. 1986).

*m-Cresol*. Williams (1938) reported that 22% of the sulfate conjugate of *m-Cresol* (in water) was excreted after 290 mg/kg *m-Cresol* was administered to rabbits by gavage.

Deichmann and Thomas (1943) reported that the urinary excretion of glucuronic acid and organic sulfates was increased in three rabbits orally dosed with *m-Cresol*. Normal daily urinary excretion of glucuronic acid in rabbits averaged about 35 mg. The dose, however, was not stated.

Bray et al. (1950) administered *m-Cresol* (in NaHCO<sub>2</sub>) to rabbits by gavage. Urinary metabolites were evaluated after administration of 500 mg of *m-Cresol*. Ten percent of the dose was excreted as ethereal sulfate, 60% as ether glucuronide, 1% as the free cresol, about 3% as 2,5-dihydroxytoluene, and a trace amount as 3,4-dihydroxytoluene.

Bardodej and Krivucova (1961) reported that 20% of a subcutaneous dose of 7.2 to 10.0 mg *m-Cresol* was excreted unchanged via urine in guinea pigs.

*o-Cresol*. Williams (1938) administered *o-Cresol* (in water) to rabbits by gavage. The extent of sulfate conjugation was determined before and after a 290 mg/kg *o-Cresol* dose. Twenty-two percent of the *o-Cresol* was excreted as the sulfate conjugate.

Deichmann and Thomas (1943) reported that the urinary excretion of glucuronic acid and organic sulfates was increased in three rabbits orally dosed with *o-Cresol*. Normal daily excretion of glucuronic acid in the urine of rabbits averaged about 35 mg. The *o-Cresol* Dose was not stated.

Bray et al. (1950) administered *o-Cresol* (in NaHCO<sub>2</sub>) to rabbits by gavage. Urinary metabolites were evaluated after administration of 500 mg of *o-Cresol*. Fifteen percent of the dose was excreted as ethereal sulfate, 72% as ether glucuronide, 1% as free cresol, and about 3% as 2,5-dihydroxytoluene.

In an in vitro study, Sato et al. (1956) incubated *o-Cresol* with rat liver slices, homogenate, and/or supernatant. Rat liver preparations metabolized *o-Cresol* to 2,5-dihydroxytoluene and only a trace amount was metabolized to 2-hydroxybenzene methanol.

Bardodej and Krivucova (1961) administered a subcutaneous dose of 7.2 to 10.0 mg *o-Cresol* to guinea pigs. About 38% of the dose was excreted unchanged via urine.

*p-Cresol*. Mareque and Marenzi (1938) administered a single dose of *p-Cresol* (8 mg) to rats by gavage. Intestinal absorption was monitored for 1 h. The absorption was slow and irregular, ranging from 0.57 to 7.55 mg/h/100 g body weight.

Williams (1938) administered *p-Cresol* (in water) to female rabbits by gavage. Urinary sulfate was evaluated before and after a 290 mg/kg *p-Cresol* dose. Approximately 15.5% of the *p-Cresol* was excreted as the sulfate conjugate.

Deichmann and Thomas (1943) reported that the urinary excretion of glucuronic acid and organic sulfates was moderately increased after rabbits were orally dosed with *p-Cresol*. Normal daily excretion of glucuronic acid in the urine of rabbits averaged about 35 mg. The *p-Cresol* dose was not stated.

Bray et al. (1950) administered *p-Cresol* (in NaHCO<sub>2</sub>) to rabbits by gavage. Urinary metabolites were evaluated after administration of 250 to 500 mg of *p-Cresol*. *p-Cresol* was more toxic

than *m-* or *o-Cresol*. *p-Cresol* was administered at 500 mg only if rabbits were given their daily food 1 to 2 h before *p-Cresol* administration. Fifteen percent of the dose was excreted as ethereal sulfate, 61% as ether glucuronide, 2% as the free cresol, about 7% as free hydroxybenzoic acid, 3% as conjugated hydroxybenzoic acid, and a trace amount of 3,4-dihydroxytoluene.

Lawrie et al. (1985) reported that adult male rats given a diet containing 7.5% saccharin for 40 days excreted about 3 times the normal amount of *p-Cresol* found in urine (2.5 mg/kg/day). In a separate study, there was a 10-fold increase in the amount of *p-Cresol* in the urine from saccharin-treated (7.5% in diet) rats at 13, 18, and 24 months. *p-Cresol* is formed by the gut flora from tyrosine. The increased excretion of *p-Cresol* confirms that microbial catabolism of amino acids other than tryptophan is affected by saccharin administration.

Parke (1968) reported that *p-Cresol* fed to rabbits was excreted in the urine as glucuronide (60%) and sulfate (15%) conjugates, about 10% was oxidized to *p*-hydroxybenzoic acid and a trace was hydroxylated to 3,4-dihydroxytoluene. The urinary metabolites from rabbits fed *p-Cresol* described by this author are diagramed in Figure 1.

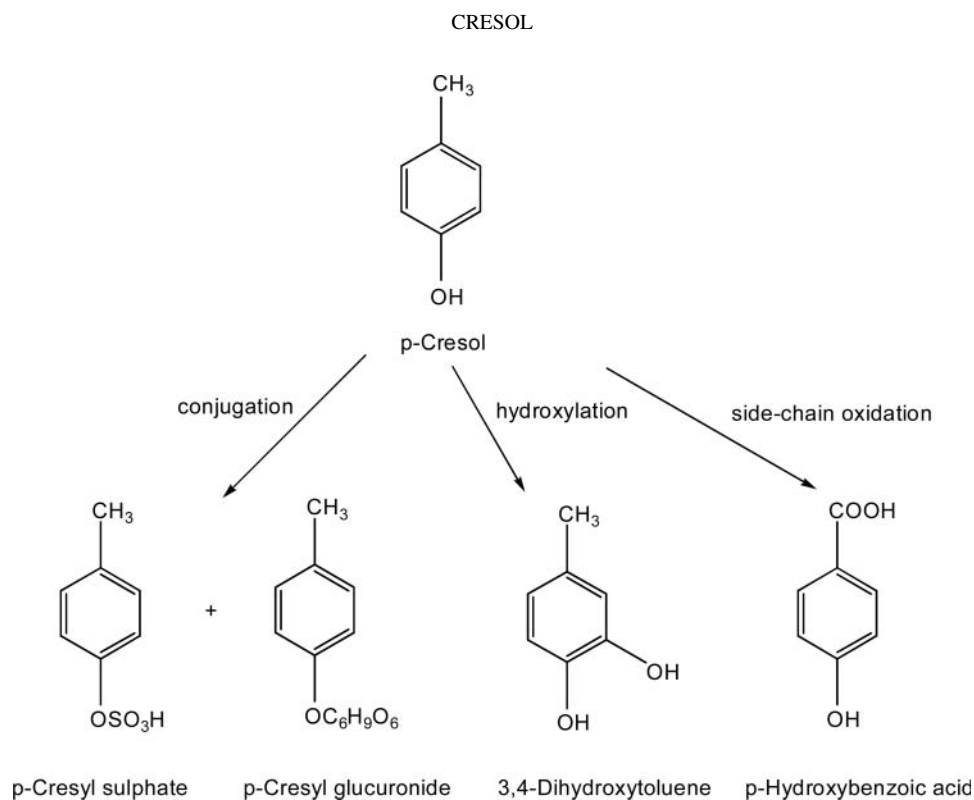
Radecki et al. (1988) investigated the production of two intestinal toxins, *p-Cresol* and ammonia, to determine which sites along the gastrointestinal tract of young pigs are the most productive in the formation of *p-Cresol* and ammonia. The in vitro microbial production of *p-Cresol* was investigated using six 21-day-old pigs fed corn-soybean meal diet. Pigs were killed 14 days later and samples were taken from each section of the gastrointestinal tract. *p-Cresol* production from *p*-hydroxyphenylacetic acid was only detected in measurable amounts in the cecum, upper colon, and lower colon.

Hinz et al. (1991) investigated the percutaneous penetration of *p-Cresol* across hairless mouse skin in vitro. The skin was obtained from female mice aged 6 to 23 weeks and was used immediately following euthanasia. The dose was 4 µg/cm<sup>2</sup>. The cumulative absorption of *p-Cresol* by hairless mouse skin was 69 ± 6%, 74 ± 4%, and 77 ± 3% over 6, 12, and 24 h respectively. The maximum flux of *p-Cresol* was 25 ± 3.6% of dose/hour.

Vanholder et al. (1999) stated that *p-Cresol* is metabolized through conjugation, mainly sulfation and glucuronidation, but removal of the unconjugated *p-Cresol* is done in part via urine. *p-Cresol* is partially lipophilic: it strongly binds to plasma protein and is retained when the kidneys fail.

*Thymol*. Schröder and Vollmer (1932) administered *Thymol* in sesame oil to three rats and two rabbits by gavage and the amount of *Thymol* in tissues, blood, urine, and feces was measured 2 to 24 h after dosing. Doses were 500 mg in rats and ranged between 1000 to 3000 mg in rabbits. *Thymol* was found in the stomach, intestines, and urine.

Robbins (1934) administered a single dose of 1 or 3 g of *Thymol* in a gelatin capsule to dogs. There were three to four dogs (weight not stated) per dose group. Urine and feces were collected for analysis. No *Thymol* was in the feces of either test

**FIGURE 1**

Urinary metabolites from rabbits fed p-Cresol (Parke 1968).

group. Thirty-four percent or 22% of the dose was recovered in urine from dogs given 1 or 3 g of Thymol, respectively.

Takada et al. (1979) studied the metabolic detoxification of Thymol in rabbits and humans. Two human volunteers were orally dosed with 0.6 g Thymol/kg body weight and the metabolites excreted in urine over 24 h were identified. Urine was filtered, fractionated, and assayed using gas chromatography and thin-layer chromatography. It was determined that any oxidized product of Thymol may be excreted into the urine in the conjugated or unconjugated form. It was concluded that Thymol may be metabolized to thymol glucuronide, thymol sulfate, and thymohydroquinone sulfate. Very small amounts of unchanged Thymol were excreted into urine.

These authors also fed three rabbits (2.76 to 2.90 kg) a standard diet plus 0.5 g/kg body weight of Thymol in a capsule. Twenty-four-hour urine specimens were collected and filtered. Twenty-four hours after Thymol administration,  $183.6 \pm 2.0$  mg of glucuronic acid and  $65.1 \pm 18.0$  mg of ethereal sulfuric acid were excreted, respectively. The main metabolite of Thymol in rabbits was thymol glucuronide in which the aglycone (noncarbohydrate group of a glycoside molecule) was intact (Takada et al. 1979).

Austgulen et al. (1987) studied the metabolism of Thymol in male albino rats (Wistar strain derived) using capillary GC-MS. The rats weighed between 250 and 350 g at the start of the experiment. Food and water were given ad libitum. The number of rats used was not stated. Thymol, in 1 to 2 ml of propylene

glycol, was dosed by gavage at 1 mmol/kg. Control animals received solvent only and urine samples were collected and stored at  $-10^{\circ}\text{C}$  at 24-h intervals.

Urine samples contained six Thymol metabolites. Thymol was the most prominent urinary component at 24 h, followed closely by the propan-1-ol derivative. Only small amounts of Thymol were detected at 48 h and no metabolites were found in the 48- to 72-h samples. The authors concluded that Thymol and its metabolites undergo rapid excretion (Austgulen et al. 1987).

**Carvacrol.** Meyer and Meyer (1959) reported on the absorption of Carvacrol in mice. Carvacrol with eserine was applied to  $2.2\text{ cm}^2$  of intact shaved abdominal skin of 6 male mice. The eserine concentration was 0.25% relative to the base. Carvacrol was not absorbed within 2 h.

Williams (1959) reported that Carvacrol appears to be slowly absorbed from rabbit intestine; 22 h after administration of 1.5 g, 25% was excreted in urine and 30% was still in the gastrointestinal tract.

Schröder and Vollmer (1932) administered Carvacrol in sesame oil to rats and rabbits by gavage and the amount of Carvacrol in tissues, blood, urine, and feces was measured at 2 to 24 h after dosing. Doses were 500 mg in rats and ranged between 1500 and 5000 mg in rabbits. Carvacrol was found in the stomach, intestines, and urine with small amounts in lung, liver, and muscle of rats. Carvacrol was found in the stomach, intestines, and urine of rabbits.

Austgulen et al. (1987) studied the metabolism of Carvacrol in male albino rats (Wistar strain derived) using capillary gas chromatography–mass spectrometry. The rats weighed between 250 and 350 g at the start of the experiment. Food and water were given ad libitum. Carvacrol, 1 mmol/kg dissolved in 1 to 2 ml of propylene glycol, was given by gavage. Control animals received solvent only. Urine samples were collected and stored at  $-10^{\circ}\text{C}$  at 24-h intervals.

Seven metabolites of Carvacrol were identified in the urine. The major chromatographic eluate was propan-1-ol derivative whereas Carvacrol was less abundant. The 48-h samples contained Carvacrol and two of its metabolites. No metabolites were detected in the 48- to 72-h sample. The authors concluded that Carvacrol and its metabolites undergo rapid excretion (Austgulen et al. 1987).

### Hepatic Effects

Male Wistar rats were dosed orally with 400 mg/kg PCMC to determine the effect on the development of hepatocellular vacuolation (Meiss et al. 1981). Hepatic tissue taken from the left lobe of the liver 7 days after dosing was examined by electron microscopy. Markedly widened intracellular spaces, intracytoplasmic vacuoles of different sizes, and alterations of the cell organelles and nuclei were observed. Cells from older animals contained more invaginations and vacuoles than those of younger animals; when younger animals were fasted prior to being killed, the vacuolation was similar to that of older animals.

The investigators considered the origin of the hepatic cell vacuoles to be from the intracellular space and observed the following stages of their formation: dilated cell surface, often limited to a restricted region; the plasmalemma formed vesicular invaginations; the invaginations extended to form large vacuoles with an open connection to intercellular space. The investigators theorized that increasing blood pressure caused by PCMC intoxication of the liver was the reason for the pathological invaginations in the region of the contacts between the hepatocytes (Meiss et al. 1981).

### Neurological Effects

A single dose of 0.2% PCMC (to correspond with 0.25 mg/kg and 0.5 mg/kg) was injected into the cisterna magna of three rabbits per dose after withdrawal of an appropriate amount of cerebrospinal fluid; two animals were used as controls (Gray and Naim 1972). One animal from each dose group was killed after 24 h, 48 h, and 5 days. None of the animals developed any functional disability suggestive of neurological damage. There were no pathological lesions due to PCMC, and the brains had no evidence of meningeal parenchymatous damage.

### Antimicrobial and Fungicidal Activity

Appropos of their use as preservatives many studies have examined the ability of these ingredients to kill bacteria and/or fungi. Table 6 summarizes these data.

### Cytotoxicity

**PCMC.** The effect of PCMC on intracellular free calcium levels in cultivated mouse myotubes was studied. Upon bath application with 250  $\mu\text{M}$  PCMC, there was a small response. Upon bath application with 500  $\mu\text{M}$  PCMC for 90 s, two separate calcium signals could be discriminated. The two signals were a transient and a sustained signal. Upon drug removal, intracellular calcium rapidly decreased to a new level that was always slightly higher than the original base line. The concentration-response curve was very steep (Gschwend et al. 1999).

***m-Cresol*, *o-Cresol*, and *p-Cresol*.** Thompson et al. (1994) compared the toxicity of *m-Cresol*, *o-Cresol*, and *p-Cresol* using precision-cut rat liver slices as a test system. Male Sprague-Dawley rats (200 to 225 g) received food and water ad libitum and were maintained on this diet at least 1 week prior to use. Some rats were pretreated with phenobarbital 4 days prior to use to test for metabolic activation. Liver cut slices were prepared from the liver cores in ice-cold Sacks buffer. Individual slices were preincubated for 30 min at  $37^{\circ}\text{C}$  in rotating 20-ml glass scintillation vials containing 2.5 ml Krebs-Hepes buffer. Following preincubation, the experiments were initiated by transferring the slices to new vials containing Krebs-Hepes buffer and one of the Cresol isomers (which were added in water or dimethylsulfoxide [DMSO] in a volume not exceeding 25  $\mu\text{l}$ ). Experiments were done in triplicate. Cytotoxicity was assessed by measuring lactate dehydrogenase (LDH) in the medium. Slices were removed from the incubator at appropriate time points and transferred to 1% Trinton X-100 solution and were stored overnight at  $4^{\circ}\text{C}$  prior to the LDH measurement.

At 2 mM, only *p-Cresol* caused toxicity compared to control incubations; over a 6-h incubation period over 90% cell death occurred in slices exposed to *p-Cresol*, compared with less than 20% in controls or those exposed to *m-Cresol* or *o-Cresol* at the same concentration.

Metabolic activation enhanced the toxicity of each isomer relative to controls. *m-Cresol* and *o-Cresol* did not show toxic signs until 5 mM, whereas *p-Cresol* caused toxic effects at 0.25 mM. In general, 5- to 10-fold greater concentrations of *m-Cresol* and *o-Cresol* were required to see similar toxic effects as compared to *p-Cresol*. The authors could not determine what *p-Cresol* intermediate caused the isomer's increased toxicity (Thompson et al. 1994).

A follow-up study by Thompson et al. (1996) determined that toxicity of *p-Cresol* was likely dependent on the formation of a reactive quinone methide intermediate.

***m-Cresol*.** Bridges et al. (1977) studied the ability of *m-Cresol* to inhibit polymorphonuclear leukocyte chemotaxis. The vehicle was either DMSO or Gay medium. *m-Cresol* weakly inhibited polymorphonuclear leukocyte chemotaxis at concentrations  $>5$  mM.

Thelestam et al. (1980) reported on the ability of *m-Cresol* to increase the permeability of human lung fibroblast membranes by measuring the release of an intracellular nucleotide marker. The human diploid embryonic lung fibroblasts were cultivated

**TABLE 6**  
Antimicrobial and fungicidal activity of Cresols

Methods/concentration/species	Results	Reference
<b>PCMC</b>		
A study was conducted to determine whether PCMC affected the enzymatic activity or the use of amino acids in the inhibition of germination of <i>Bacillus subtilis</i> spores.	The initiation of germination of <i>B. subtilis</i> in complete medium and in phosphate buffer plus the enzyme subtilopeptidase is inhibited by PCMC, but the activity of the enzyme is not, suggesting that the proteolytic enzyme is not the primary site of inhibition. Amino acid-initiated germination was reversibly inhibited by PCMC, suggesting that the inhibitor prevents the initiation of germination of bacterial spores at the level of amino acid use, both in complete medium and with subtilopeptidase.	Sierra 1970
The antimicrobial spectrum of 0.02% PCMC included gram-positive and gram-negative bacteria, including tubercle bacilli, and also yeasts and fungi.	Antimicrobial effects observed.	Paulus and Genth 1983
The effectiveness of PCMC (0.1%) as a preservative in aqueous cream was examined using the Test for Efficacy of Preservatives (British Pharmacopoeia, 1982) modified to include membrane filtration. Ten g samples of the cream were inoculated with 0.1 ml of a suspension containing either <i>Candida albicans</i> or <i>Aspergillus niger</i> to give concentrations of approximately 10 <sup>6</sup> organisms/g.	PCMC met the British Pharmacopoeia requirements for the efficacy of antimicrobial preservatives [the number of molds or yeasts recovered per gram is reduced by a factor of not less than 100 within 14 days of challenge and there is no increase thereafter] when tested against <i>A. niger</i> , but not when tested against <i>C. albicans</i> , using membrane filtration to isolate the microorganisms.	Brown et al. 1986
<b>m-Cresol</b>		
m-Cresol at a concentration of 1000 ppm.	Reduced the growth of <i>Escherichia coli</i> by about 50% with no effect on cell size	Loveless et al. 1954
m-Cresol (0.5% in ethanol) against <i>Aspergillus fumigatus</i> , <i>Microsporum canis</i> , <i>Trichophyton mentagrophytes</i> TM-4, <i>Trichophyton mentagrophytes</i> TM-1, <i>Trichophyton rubrum</i> , <i>Candida albicans</i> , and <i>Candida crusei</i> .	Antifungal activity observed.	Hejtmánková et al. 1979
m-Cresol (1%) in a vehicle of n-propanol and ethylene glycol monomethyl ether.	No antifungal effects in 9 strains tested (up to 7 day incubation). There were no antibacterial effects in 6 strains tested (up to 48 h incubation). Specific strains were not stated.	RIFM 2001b
m-Cresol (0.1% and 1%) was purified by fractional distillation and tested in <i>B. subtilis</i> , <i>S. enteritidis</i> , <i>S. aureus</i> , <i>P. aeruginosa</i> , <i>P. morgani</i> , and <i>E. coli</i> . Antibacterial activity was assessed by the agar streak method.	There was growth inhibition of <i>P. aeruginosa</i> at a 0.1% dilution and all other bacteria exposed to 1% dilutions of m-Cresol had inhibited growth.	Katayama and Nagai 1960

(Continued on next page)

**TABLE 6**  
Antimicrobial and fungicidal activity of Cresols (*Continued*)

Methods/concentration/species	Results	Reference
<i>o</i> -Cresol		
<i>o</i> -Cresol (2 mM) in fungus (unstated species).	Inhibited fungal growth at a concentration of 2 mM.	Bollag and Shuttleworth 1988
<i>o</i> -Cresol at a concentration of 0.5% (in ethanol) against <i>Microsporum canis</i> , <i>Trichophyton mentagrophytes</i> TM-4, <i>Trichophyton mentagrophytes</i> TM-1, <i>Trichophyton rubrum</i> , <i>Candida albicans</i> , and <i>Candida crusei</i> .	Produced antifungal activity.	Hejtmánková et al. 1979
<i>o</i> -Cresol (1%) in a vehicle of n-propanol and ethylene glycol monomethyl ether.	Had no antifungal effects in 9 strains tested (up to 7 day incubation). There were antibacterial effects in 2 of 6 strains tested (up to 48 h incubation).	RIFM 2001c
<i>o</i> -Cresol was purified by fractional distillation and tested in <i>B. subtilis</i> , <i>S. enteritidis</i> , <i>S. aureus</i> , <i>P. aeruginosa</i> , <i>P. morganii</i> , and <i>E. coli</i> . Antibacterial activity was assessed by the agar streak method.	There was growth inhibition of <i>P. aeruginosa</i> and <i>P. morganii</i> at a 0.1% dilution and all other bacteria exposed to 1% dilutions of <i>o</i> -Cresol had inhibited growth.	Katayama and Nagai 1960
<i>p</i> -Cresol		
<i>p</i> -Cresol (2 mM) against fungus (unstated species).	Inhibited fungal growth at a concentration of 2 mM and no toxicity was observed.	Bollag and Shuttleworth 1988
<i>p</i> -Cresol at a concentration of 0.5% (in ethanol) against <i>Microsporum gypseum</i> , <i>Microsporum canis</i> , <i>Trichophyton mentagrophytes</i> TM-4, <i>Trichophyton mentagrophytes</i> TM-1, <i>Trichophyton rubrum</i> , <i>Candida albicans</i> , and <i>Candida crusei</i> .	Produced antifungal activity.	Hejtmánková et al. 1979
<i>p</i> -Cresol was purified by fractional distillation and tested in: <i>B. subtilis</i> , <i>S. enteritidis</i> , <i>S. aureus</i> , <i>P. aeruginosa</i> , <i>P. morganii</i> , and <i>E. coli</i> . Antibacterial activity was assessed by the agar streak method.	There was growth inhibition of <i>S. aureus</i> at a 5% dilution and all other bacteria exposed to 1% dilutions of <i>p</i> -Cresol had inhibited growth.	Katayama and Nagai 1960
Thymol		
<i>Aspergillus flavus</i> , <i>Asperigillus niger</i> , <i>Geotrichum candidum</i> , 2 nonidentified <i>Mucor</i> spp., <i>Penicillium roqueforti</i> , and 3 non-identified <i>Penicillium</i> spp.). pH of 5.5 and at concentrations of 0.025% and 0.05% (w/v) grown on potato dextrose agar at 25°C.	Almost complete inhibition of growth of nine foodborne fungi.	Akgül and Kivanc 1988
Thymol against mycelial growth of <i>A. flavus</i> and <i>A. niger</i> .	Complete inhibition of mycelial growth at 1000 ppm and greater.	Tripathi et al. 1986
Growth inhibition and lethal effects of synthetic Thymol were investigated using a food spoilage yeast, <i>Debaomyces hansenii</i> . Inhibitory activity was studied at 25, 50, 75, and 100 ppm over a 55-h incubation period. Lethality was examined using Thymol at 100 and 125 ppm over a 4-day incubation period.	The minimum concentration of Thymol for total growth inhibition was 100 ppm. Thymol had little effect on initial culture outgrowth up to 75 ppm. Lethality was observed with Thymol at 100 and 125 ppm concentration over a 4-day incubation.	Curtis et al. 1996

(*Continued on next page*)

**TABLE 6**  
Antimicrobial and fungicidal activity of Cresols (*Continued*)

Methods/concentration/species	Results	Reference
Thymol (125 to 500 $\mu\text{g/ml}$ ) against 4 <i>Streptococcus</i> strains, 3 <i>Prevotella</i> strains, and <i>Peptostreptococcus anerobbius</i> (ATCC 14956).	Thymol had antimicrobial activity against 8 oral bacteria at minimum inhibitory concentrations (MICs) between 125 to 500 $\mu\text{g/ml}$ .	Didry et al. 1994
Thymol at 100–175 $\mu\text{g/ml}$ against <i>Salmonella typhimurium</i> under aerobic and anaerobic conditions. Thymol at 175 to 250 $\mu\text{g/ml}$ against <i>Staphylococcus aureus</i> under aerobic and anaerobic conditions.	Thymol at 100 $\mu\text{g/ml}$ did not affect the growth of <i>Salmonella typhimurium</i> ; viable counts decreased when 140 $\mu\text{g/ml}$ of Thymol was added, and at 175 $\mu\text{g/ml}$ no viable salmonella were detected. Efficacy more pronounced under anaerobic conditions. Thymol showed antagonistic effects against <i>Staphylococcus aureus</i> in the range of 175 to 250 $\mu\text{g/ml}$ , the effects were equally pronounced under aerobic and anaerobic conditions. Thymol did not show a progressive increase in antibacterial effect with increased concentration, instead at a critical concentration a sudden reduction in viable counts was observed.	Juven et al. 1994
Thymol's antifungal activity was tested against the fungi: <i>F. moniliforme</i> , <i>R. solani</i> , <i>S. sclerotiorum</i> , and <i>P. capsici</i> at concentrations of 50, 100, 150, 200, and 250 $\mu\text{g/ml}$ .	Mycelial growth inhibition ranged from 0% to 81.9% at the lowest level and was 100% at all Thymol concentrations above 100 $\mu\text{g/ml}$ .	Muller-Riebau et al. 1995
Thymol against 25 different periodontopathic bacteria and strains at MICs between 39 to 625 $\mu\text{g/ml}$ .	Had antimicrobial activity. There was no significant difference in the antibacterial activity of Carvacrol and Thymol because they are structural isomers.	Osawa et al. 1990
The efficacy of Thymol and Carvacrol was tested against 5 <i>Aspergillus</i> species, 4 <i>Penicillium</i> species, and 2 <i>Fusarium</i> species, all of which normally contaminate food.	Thymol appeared to be less efficient than Carvacrol yet still very potent.	Pauli and Knobloch 1987
Thymol against growth of 3 <i>Penicillium</i> species in agar after 3 days of incubation.	Had a strong and intense fungicidal effect.	Scora and Scora 1998
Thymol concentrations from 0.02% to 0.05% against <i>Listeria monocytogenes</i> and <i>Bacillus subtilis</i> over 19 h.	Significantly reduced cell growth when compared to untreated control cells.	Ettayebi et al. 2000
Investigated the influence of pH on the fungitoxic activity of Thymol in 8 strains of <i>Aspergillus</i> . Test concentrations of Thymol in broth were 0.1, 0.5, and 1.0 mM. A spore suspension of the test fungus was incubated for 7 days at 27°C. Three replicates per treatment were used and the experiments were repeated twice.	Mycelial growth in all strains was completely inhibited at pH 4, 6, or 8 in the presence of 1.0 mM Thymol. At 0.1 or 0.5 mM Thymol, there was partial mycelial growth at all pH levels, but significantly less growth at pH 4 and 8.	Thompson 1990

(Continued on next page)

**TABLE 6**  
Antimicrobial and fungicidal activity of Cresols (*Continued*)

Methods/concentration/species	Results	Reference
Thymol (25–150 µg/ml) against 2 strains of <i>Aspergillus parasiticus</i> (CBS 26027 and NRRL 2999) at 30°C.	Thymol (150 µg/ml) was effective in inhibiting growth of 2 strains of <i>Aspergillus parasiticus</i> (CBS 26027 and NRRL 2999) at 30°C. Thymol (25 µg/ml) caused growth and aflatoxin inhibition in the <i>A. parasiticus</i> strain CBS 26027. However, in <i>A. parasiticus</i> strain NRRL 2999, Thymol (25 µg/ml) caused stimulation in growth and aflatoxin accumulation at lower doses.	Karapinar 1990
Thymol (150–1000 ppm) against growth of <i>Aspergillus flavus</i> .	Thymol completely inhibited the growth of <i>Aspergillus flavus</i> and prevented any formation of aflatoxin at 1000 ppm. Thymol had a MIC of 150 ppm.	Mahmoud 1994
Thymol (2%) against <i>Aspergillus flavus</i> in maize grain.	Thymol reduced <i>Aspergillus flavus</i> contamination of maize grain by 52.3%.	Montes-Belmont and Carvajal 1998
Thymol against <i>E. coli</i> (2 strains), <i>S. typhimurium</i> , 5 gram-positive bacteria, and 2 species of yeast.	The minimum bactericidal concentrations (MBCs) of Thymol determined in <i>E. coli</i> , <i>S. typhimurium</i> , gram-positive bacteria, and yeast were 225 to 450, 56.25, 225 to 450, and 112.5 µg/ml, respectively.	Cosentino et al. 1999
Thymol (10% in poppyseed oil) against <i>Staphylococcus aureus</i> .	10% Thymol had positive effects on an emulsion of <i>Staphylococcus aureus</i> . Sterility was obtained in 30 to 60 min.	RIFM 2001e
The antibacterial activity of Thymol (50% in ethanol) was studied using 15 oral bacteria in a microtitre plate assay and a broth dilution assay.	The Thymol MICs ranged from 0.02% to 0.06% in the microtitre plate assay and from 0.01% to 0.03% in the broth dilution assay.	Shapiro et al. 1994
Thymol was purified by fractional distillation and tested in <i>B. subtilis</i> , <i>S. enteritidis</i> , <i>S. aureus</i> , <i>P. aeruginosa</i> , <i>P. morganii</i> , and <i>E. coli</i> . Antibacterial activity was assessed by the agar streak method.	There was growth inhibition of <i>S. enteritidis</i> , <i>P. aeruginosa</i> , and <i>E. coli</i> at a 0.05% dilution and all other bacteria exposed to 0.1% dilutions of Thymol had inhibited growth.	Katayama and Nagai 1960
<i>o</i> -Cymen-5-ol		
<i>o</i> -Cymen-5-ol against <i>Pseudomonas aeruginosa</i> , <i>Salmonella typhosa</i> ( <i>Bacillus typhi</i> ), <i>Escherichia coli</i> , and <i>Staphylococcus aureus</i> .	Demonstrated antimicrobial activity.	Hayami et al. 1977; Tanaka and Hiramatsu 1953
The phenol coefficient of <i>o</i> -Cymen-5-ol.	Phenol coefficient of <i>o</i> -Cymen-5-ol was 19 for <i>E. coli</i> and 17 for <i>S. typhosa</i> and <i>S. aureus</i> .	Tanaka and Hiramatsu 1953
<i>o</i> -Cymen-5-ol against bacteria (0.01 to 0.02%) and fungi (0.01 to 0.05%).	Inhibited the growth of bacteria at concentrations of 0.01% to 0.02%, and inhibits the growth of fungi and yeasts at concentrations of 0.01% to 0.05%.	Kitazawa 1955
Carvacrol		
Carvacrol at a pH of 5.5 and at concentrations of 0.025% and 0.05% (w/v) on potato dextrose agar at 25°C against nine foodborne fungi ( <i>Aspergillus flavus</i> , <i>Aspergillus niger</i> , <i>Geotrichum candidum</i> ,	Almost complete growth inhibition.	Akgül and Kivanc 1988

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**TABLE 6**  
Antimicrobial and fungicidal activity of Cresols (*Continued*)

Methods/concentration/species	Results	Reference
2 nonidentified <i>Mucor</i> spp., <i>Penicillium roqueforti</i> , and 3 nonidentified <i>Penicillium</i> spp.).		
An essential oil derived from <i>Thymus capitatus</i> containing 90% Carvacrol and 10% Thymol was tested at concentrations: 10, 50, 100, 1000, and 5000 µg/ml.	The essential oil inhibited the growth of <i>Staphylococcus aureus</i> , <i>Streptococcus pyogenes</i> , <i>Escherichia coli</i> , <i>Salmonella typhimurium</i> , <i>Klebsiella</i> spp., <i>Pseudomonas aeruginosa</i> , and <i>Corynebacterium pyogenes</i> (percent of inhibition increased with concentration of essential oil).	Kandil et al. 1994
Carvacrol (500 ppm) against <i>Aspergillus flavus</i> and <i>Aspergillus parasiticus</i> at 28°C.	Carvacrol (500 ppm) complete growth and aflatoxin production inhibition of <i>Aspergillus flavus</i> and <i>Aspergillus parasiticus</i> at 28°C. Increased growth inhibition was observed at 15°C. <i>A. flavus</i> was more sensitive to Carvacrol than <i>A. parasiticus</i> . At 50 and 100 ppm, Carvacrol partially inhibited growth and still markedly inhibited aflatoxin production.	Akgül et al. 1991
Carvacrol against a food spoilage yeast, <i>Debomyces hansenii</i> . Inhibitory activity was studied at 25, 50, 75, and 100 ppm over a 55-h incubation period. Lethality was examined using Carvacrol at 100 and 125 ppm over a 4-day incubation period.	The minimum concentration of Carvacrol for total inhibition was 100 ppm. Carvacrol had little effect on initial culture outgrowth up to 75 ppm. Lethality was observed with Carvacrol at 100 and 125 ppm concentrations.	Curtis et al. 1996
Carvacrol against 8 oral bacteria (4 <i>Streptococcus</i> strains, 3 <i>Prevotella</i> strains, and <i>Peptostreptococcus anerobbius</i> ATCC 14956).	Antimicrobial activity reported, MICs between 125 to 500 µg/ml.	Didry et al. 1994
Carvacrol is one of the main active ingredients in <i>Micromeria nervosa</i> (Labiatae). Thymol and Carvacrol were isolated from <i>M. nervosa</i> .	No significant difference in antimicrobial activity between Thymol and Carvacrol.	Ali-Shtayeh et al. 1997
Carvacrol (200 ppm and greater) against <i>Salmonella typhimurium</i> .	Reduced the viable counts of <i>S. typhimurium</i> at concentrations of 200 µg/ml of Carvacrol and greater. Small differences in efficacy were noted under aerobic and anaerobic conditions. Carvacrol did not have a progressive increase in antibacterial effect with increased concentration, instead at a critical concentration a sudden reduction in viable counts was observed.	Juven et al. 1994
Carvacrol antibacterial activity against <i>Salmonella typhimurium</i> .	MIC of 250 µg/ml.	Kim et al. 1995a
Carvacrol activity against <i>Escherichia coli</i> , <i>E. coli</i> O157:H7, <i>Salmonella typhimurium</i> , <i>Listeria monocytogenes</i> , and <i>Vibrio vulnificus</i> .	MICs of <i>E. coli</i> , <i>E. coli</i> O157:H7, <i>Salmonella typhimurium</i> , <i>Listeria monocytogenes</i> , and <i>Vibrio vulnificus</i> were 500, 500, 500, 250, and 250 µg/ml, respectively.	Kim et al. 1995b

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**TABLE 6**  
Antimicrobial and fungicidal activity of Cresols (*Continued*)

Methods/concentration/species	Results	Reference
Carvacrol (both pure and in savory extract 10–455 ppm) against <i>Cladosporium herbarum</i> and <i>Penicillium glabrum</i> after 7 and 14 days.	Carvacrol had activity against <i>Cladosporium herbarum</i> at concentrations of 10, 185, and 455 ppm and <i>Penicillium glabrum</i> at concentrations of 100, 120, 370, and 455 ppm after 7 and 14 days with growth inhibition ranging from 28.6% to 100%.	Martini et al. 1996
The antimicrobial activity of Carvacrol (50–250 µg/ml) was tested against the fungi <i>F. moniliforme</i> , <i>R. solani</i> , <i>S. sclerotium</i> , and <i>P. capisci</i> .	Mycelial growth inhibition ranged from 0% to 80.3% at the lowest dose and was 100% at all Carvacrol doses above 100 µg/ml.	Muller-Riebau et al. 1995
Carvacrol's antimicrobial activity against 25 different periodontopathic bacteria and strains was tested.	MIC between 39 to 625 µg/ml. The authors stated that there was no significant difference in the antibacterial activity of Carvacrol and Thymol because they are structural isomers.	Osawa et al. 1990
Carvacrol (50 µl) against <i>E. coli</i> O157:H7 colony formation after 48 h.	Not affected by 50 µl of Carvacrol after 48 h. The authors hypothesized that the low concentration of Carvacrol may have accounted for its ineffectiveness.	Park et al. 2000
The efficacy of Carvacrol was tested against 5 <i>Aspergillus</i> species, 4 <i>Penicillium</i> species, and 2 <i>Fusarium</i> species, all of which normally contaminate food.	Thymol was also studied, Thymol appeared to be a less efficient growth inhibitor yet still very potent in comparison to Carvacrol.	Pauli and Knobloch 1987
Carvacrol against 3 <i>Penicillium</i> species in agar after 3 days of incubation.	Carvacrol had a strong and intense inhibition of the growth of 3 <i>Penicillium</i> species in agar after 3 days of incubation.	Scora and Scora 1998
Investigated the influence of pH on the fungitoxic activity of Carvacrol using 8 strains of <i>Aspergillus</i> . Test concentrations of Carvacrol in broth were 0.1, 0.5, and 1.0 mM. A spore suspension of the test fungus was incubated for 7 days at 27°C. Three replicates per treatment were used and the experiments were repeated twice.	Mycelial growth in all strains was completely inhibited at pH 4 and 8 in the presence of 1.0 mM Carvacrol. At 0.1 or 0.5 mM Carvacrol, there was partial mycelial growth at all pH levels, but significantly less growth at pH 4 and 8.	Thompson 1990
Carvacrol (1000 ppm) against fungal growth and aflatoxin production by <i>Aspergillus flavus</i> .	Reported Carvacrol (1000 ppm) inhibited fungal growth and aflatoxin production by <i>Aspergillus flavus</i> by 24.6% and 50%, respectively.	Mahmoud 1994
1 to 3 mM Carvacrol against <i>Bacillus cereus</i> .	Incubation for 30 min in the presence of 1 to 3 mM Carvacrol reduced the viable cell numbers of <i>Bacillus cereus</i> exponentially.	Ultee et al. 1999
Carvacrol against <i>E. coli</i> (2 strains), <i>S. typhimurium</i> , 5 gram-positive bacteria, and 2 species of yeast was investigated.	The MBC for <i>E. coli</i> , <i>S. typhimurium</i> , gram-positive bacteria, and yeast were 225, 225, 225–900, and 112.5 µg/ml, respectively.	Cosentino et al. 1999

to a cell density of  $10^5$  cells/cm<sup>2</sup>. They were then labeled with [<sup>3</sup>H]uridine. The labeled cultures were incubated with 25 mM of *m*-Cresol (in Tris-buffer saline) for 30 min at 37°C. Treatment with 0.06 M sodium borate buffer and scraping with a rubber policeman ruptured the cell membranes while leaving the nuclei

intact. Thirty-two percent of the nucleotide was released. No other details were available.

Chen et al. (1984) tested the ability of *m*-Cresol to inhibit metabolic cooperation in Chinese hamster lung fibroblast cells (wild-type 6tGs, HGPRT<sup>+</sup>; mutant 6tGr, HGPRT<sup>−</sup>) at various

concentrations (exact concentrations not stated). The ability to inhibit metabolic cooperation was indicated by the number of colonies recovered over the negative control. A positive chemical response was defined as a dose-response effect, with a recovery at least two times the negative control. *m*-Cresol was negative in the V79 cells in the metabolic cooperation assay.

*o*-Cresol. Bridges et al. (1977) studied the ability of *o*-Cresol to inhibit polymorphonuclear leukocyte chemotaxis. The vehicle was either DMSO or Gay medium. *o*-Cresol weakly inhibited polymorphonuclear leukocyte chemotaxis doses at doses >5 mM.

Garrett and Lewtas (1983) studied the cellular toxicity effects in Chinese hamster ovary (CHO) cells in culture for 20 h with *o*-Cresol at a concentration of 1000  $\mu\text{g/ml}$ . Cell samples were assayed for protein and DNA synthesis, ATP, cell number, and viability. Replicate cultures were used to calculate mean values.

CHO cell viability was 35% of control values; ATP, DNA synthesis, and protein synthesis were 8%, 0%, and 0% of control values, respectively. Estimates of the sample concentration necessary to produce a 50% response were calculated for DNA and protein synthesis. The  $\text{EC}_{50}$  of *o*-Cresol for DNA synthesis and protein synthesis was 52  $\mu\text{g/ml}$  (95% confidence intervals from 41 to 65  $\mu\text{g/ml}$ ) and 131  $\mu\text{g/ml}$  (95% confidence intervals 108 to 157  $\mu\text{g/ml}$ ), respectively. Protein synthesis appeared to be the most sensitive indicator of cellular toxicity. The authors considered *o*-Cresol a moderate toxicant in vitro, which reasonably correlated with whole-animal test data (RTECS rat oral  $\text{LD}_{50}$  for *o*-Cresol is 121 mg/kg) (Garrett and Lewtas 1983).

*p*-Cresol. Bridges et al. (1977) studied the ability of *p*-Cresol to inhibit polymorphonuclear leukocyte chemotaxis. The vehicle was either DMSO or Gay medium. *p*-Cresol weakly inhibited polymorphonuclear leukocyte chemotaxis at concentrations >5 mM.

Battelle's Columbus Laboratories (1978) employed a pre-screen confluency cytotoxicity assay to establish the toxicity dose range of *p*-Cresol in WI-38 human embryonic fibroblasts and C3H10T1/2 mouse fibroblasts. The  $\text{EC}_{50}$  values of *p*-Cresol were 0.31 mg/plate in human fibroblasts and 8.2 mg/plate in mouse fibroblasts.

Thymol. Singh (1980) reported on the effect of membrane stabilizers (Thymol) and cytochalasin-B on amylase release from dissociated mouse pancreatic acinar cells stimulated by glucagon. Thymol concentrations from  $10^{-7}$  to  $10^{-4}$  M did not alter basal release of amylase or LDH; however, at  $10^{-3}$  M, Thymol lysed dissociated acinar cells, which was indicated by an increase in amylase secretion and LDH release by 315%.

Manabe et al. (1987) clarified the method of cell damage by Thymol using rat erythrocytes, hepatocytes, and dipalmitoyl phosphatidylcholine (DPPC)-liposomes. Male, Sprague-Dawley rats (160 to 170 g), about 6 weeks old, were used for hypotonic hemolysis studies. The blood was centrifuged for 15 min and the erythrocyte-rich pellet was rinsed three times with isotonic phosphate-buffered saline. The erythrocyte concentration was adjusted to  $1 \times 10^9$  cells/ml and kept at  $0^\circ\text{C}$  before use. Thy-

mol was dissolved in DMSO and introduced to the hypotonic buffer solution to produce a DMSO concentration less than 1% of volume which previously was found to have no effect on hemolysis. Aliquots (4.3 ml) of the hypotonic buffer solution containing various concentrations of Thymol and aliquots (0.2 ml) of the erythrocyte suspension were separately preincubated at  $37^\circ\text{C}$  for 5 min, then mixed and incubated for 1 h in a  $37^\circ\text{C}$  water bath. The suspension was centrifuged and the free hemoglobin in the supernatant was determined at 540 nm. Thymol showed biphasic effects on hypotonic hemolysis. At 0.06 to 1 mM, Thymol protected erythrocytes, and at 2 to 4 mM there was a lytic effect on the erythrocytes. One millimolar of Thymol had the maximal protection for erythrocytes.

These authors also used male, Sprague-Dawley rats (220 to 250 g), about 7 weeks old, for cytotoxicity and morphological studies on isolated hepatocytes. The liver was perfused with Hepes buffer containing 0.05% (v/v) collagenase and 100 mM  $\text{CaCl}_2$  at a pressure of 40 cm  $\text{H}_2\text{O}$  for 45 min. The liver was removed, transferred to a beaker containing Hepes buffer, and split. The resulting cell suspension was filtered; the pellet was rinsed twice in Eagle's minimum essential medium solution and resuspended to a final concentration of  $2 \times 10^5$  cells/ml.

In hepatocytes, Thymol caused a significant increase in glutamic oxaloacetic transaminase (GOT) leakage at 0.2 to 4 mM. Thymol caused no change in glutamic pyruvate transaminase (GPT) or LDH leakage from controls. Thymol had a large effect on membrane fluidity because it depressed the phase-transition temperature by  $33.3^\circ\text{C}$  at 1 mM. Thymol also decreased surface tension from 72 to 53 dyne/cm at a concentration of 1 mM (Manabe et al. 1987).

Suzuki et al. (1987) examined Thymol- and 12-*O*-tetradecanoyl phorbol 13-acetate (TPA)-induced  $\text{O}_2^-$  production in the leukocytes of humans, apes, baboons, and macaque monkeys. Leukocytes were obtained from 10 ml of heparinized venous blood by dextran sedimentation and hypotonic hemolysis. Isolated leukocytes were suspended at  $2 \times 10^6/\text{ml}$  in Hank's balanced salt solution containing bovine serum albumin (BSA). Reaction mixtures contained  $2 \times 10^6$  leukocytes in 1 ml of Hank's balanced salt solution with BSA and preincubated with 50  $\mu\text{M}$  ferricytochrome *c*. The effect of superoxide dismutase and KCN on the inhibition reduction of ferricytochrome *c* in Thymol-stimulated leukocytes was also examined.

Human leukocytes were stimulated by Thymol to produce  $\cdot\text{O}_2^-$  in a directly concentration-dependent manner between 0.75 and 2 mM and the rate remained constant at a concentration greater than 2 mM. *m*-Cresol was tested as a control and was inactive, suggesting that the *o*-position of Thymol is necessary for induction. Superoxide dismutase (0.05 mg/ml) completely inhibited the reduction of ferricytochrome *c* in Thymol-stimulated leukocytes, whereas 1 mM of KCN had no effect. The authors indicated that ferricytochrome *c* reduction is due to the  $\cdot\text{O}_2^-$  release upon the respiratory burst. Leukocytes of chimpanzee and baboon cells had only 35% of the maximal  $\cdot\text{O}_2^-$  production rate obtained in human cells exposed to Thymol. However, when

cells were stimulated by TPA, no significant difference in the  $\cdot\text{O}_2^-$  production rate was observed between human and monkey cells (except chimpanzee). Human leukocytes were most responsive to Thymol among the primates tested (Suzuki et al. 1987).

In a similar study, Suzuki and Furita (1988) investigated the activation mechanism of  $\cdot\text{O}_2^-$  production in Thymol-activated neutrophils. Neutrophils ( $2.5\text{--}3 \times 10^8$  cells/animal) were obtained from the peritoneal cavity of male, adult guinea pigs (350 to 450 g) 16 to 24 h after intraperitoneal injection of 1% glycogen in isotonic saline. Cell preparations contained approximately 90% neutrophils. Thymol's ability to stimulate  $\cdot\text{O}_2^-$  release was measured by the reduction of ferricytochrome *c*. The reversibility of activation was studied. The responsiveness of Thymol-pulsed cells to trifluoperazine (TFP) and TPA was investigated. ATP content was measured using the firefly luciferase system to assess changes in intracellular ATP content by Thymol exposure.

Thymol (1 mM) stimulated  $\cdot\text{O}_2^-$  production in guinea pig neutrophils about 30 s after the addition of Thymol which continued at a constant rate for more than 5 min. The rate was independent of extracellular  $\text{Ca}^{2+}$ . Thymol-induced activity was completely inhibited by 10  $\mu\text{M}$  TFP, which is an inhibitor of protein kinase C, and its  $\text{IC}_{50}$  was 4  $\mu\text{M}$ , which was less than one-third that of TPA-induced activity. After complete activation,  $\cdot\text{O}_2^-$  production was reversed by TFP addition or by rinsing out and resuspending in a stimuli-free medium. The authors suggested that unknown protein kinases, different from protein kinase C, were involved in the Thymol-induced activation system in neutrophils. It was determined that control cells contained 6.7 nmol of ATP/107 cells, whereas Thymol-pulsed cells contained half of this concentration. During Thymol stimulation, the ATP content of control cells decreased, but Thymol-pulsed cells remained constant. These data suggest the magnitude of Thymol-induced  $\cdot\text{O}_2^-$  production in neutrophils is dependent on the initial density of the binding sites of the cells with Thymol and the initial intracellular ATP concentration (Suzuki and Furita 1988).

Arai (1988) studied the cytotoxicity of Thymol in cultured mammalian cells. Growth of V79 cells was unaffected by treatment with Thymol at 30 to 100  $\mu\text{g}/\text{ml}$  for 24 to 48 h. Complete inhibition of growth was observed at 300  $\mu\text{g}/\text{ml}$  and cell growth inhibition was significantly different between 100 and 300  $\mu\text{g}/\text{ml}$  Thymol for 24 to 48 h of treatment. Treatment with 30 to 100  $\mu\text{g}/\text{ml}$  of Thymol for 2 to 24 h had no inhibitory effect; however, 300  $\mu\text{g}/\text{ml}$  was completely cytotoxic. Thymol at 30 to 300  $\mu\text{g}/\text{ml}$  for 2 h resulted in partial inhibition of DNA, RNA, and protein synthesis in a dose-related manner.

Stammati et al. (1999) evaluated the cytotoxicity of Thymol using two viability tests, neutral red uptake (NRU) and total protein content (TPC); Hep-2 cell line derived from human larynx carcinoma was used. Thymol was tested at concentrations between 0.25 and 2.20 mM in the NRU viability test. Treated cells were examined for morphological alterations. Cells were also examined for colony-forming ability after treatment for 48 h with Thymol, and a 7-day incubation period.

Dose-dependent inhibition of NRU and TPC were observed in Hep-2 cells with different concentrations of Thymol ( $\text{IC}_{50}$ : NRU 0.71 mM and TPC 0.78 mM). The Thymol-treated cells produced a typical necrotic pattern, with cytoplasmic extrusion and no uptake of dye. Thymol did not induce apoptosis at any concentration tested. Colony-forming ability was inhibited in a dose-dependent manner; the  $\text{IC}_{50}$  of Thymol was 0.41 mM. The in vitro cytotoxicity was considered moderate (Stammati et al. 1999).

*Carvacrol.* Stammati et al. (1999) evaluated the cytotoxicity of Carvacrol using two viability tests, NRU and TPC; Hep-2 cell line was used. Carvacrol was tested at concentrations between 0.03 and 3.12 mM in the NRU viability test. Treatment cells were examined for morphological alterations. After treatment for 48 h with Carvacrol, and a 7-day incubation period, cells were examined for colony-forming ability.

Concentration-dependent inhibition of NRU and TPC were observed in Hep-2 cells with different concentrations of Carvacrol ( $\text{IC}_{50}$ : NRU 0.32 mM and TPC 0.30 mM). Carvacrol "induced the fragmentation of nuclei which is typical for condensed apoptotic phenotype and was observed at each concentration tested." Colony-forming ability was inhibited in a dose-dependent manner, the  $\text{IC}_{50}$  of Carvacrol was 0.22 mM. The in vitro cytotoxicity was considered moderate by the researchers (Stammati et al. 1999).

### Antioxidant Activity

Kamataki et al. (1978) studied the effect of Thymol on lipid peroxidation using a reconstituted lipid peroxidation system containing NADPH-cytochrome *c* reductase isolated from detergent-solubilized rat liver microsomes. There was strong inhibition of the NADPH-cytochrome *c* reductase mediated lipid peroxidation at 250 and 500  $\mu\text{M}$ .

Jimenez et al. (1993) reported clear antihepatotoxic activity in female Swiss OFF1 mice (25 to 30 g body weight) given 125 mg/kg Thymol prior to a single intraperitoneal dose of carbon tetrachloride. Serum glutamic pyruvate transaminase and hepatic malondialdehyde levels were measured.

In an evaluation of possible food antioxidants, Aeschbach et al. (1994) assessed the antioxidant actions of Thymol and Carvacrol. Both Thymol and Carvacrol decreased peroxidation of phospholipid liposomes in the presence of iron (III) and ascorbate. Thymol and Carvacrol were not able to accelerate DNA damage in the bleomycin-Fe(III) system.

Thymol and Carvacrol antioxidant activities were investigated using human aortic endothelial cells to mediate the oxidation of low-density lipoprotein (LDL) over a 12-h incubation period. Both Thymol and Carvacrol at concentrations from 1.25 to 10  $\mu\text{M}$  inhibited LDL oxidation in a concentration-dependent manner (Pearson et al. 1997).

Oguri et al. (1998) investigated the efficacy of Thymol to suppress formation of mutagenic and carcinogenic heterocyclic amines (HCAs). The HCAs 2-amino-3,8-

dimethylimidazo[4,5-f]quinoxaline (MeIQx) and 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) were studied because they are considered to be probable human carcinogens. The effects of Thymol on MeIQx formation was examined by the addition of 0.2 mmol of Thymol to a mixture of creatine (0.4 mmol), glycine (0.4 mmol), and glucose (0.2 mmol), and heating to 128°C for 2 h. L-Phenylalanine replaced glycine in the mixture to assess PhIP formation.

Thymol had no effect on MeIQx formation and exerted no appreciable effect on PhIP formation at the concentration tested (Oguri et al. 1998).

Alam et al. (1999) investigated the protective action of Thymol against carbon tetrachloride hepatotoxicity in mice. Male Swiss albino mice (25 to 27 g) were used. Carbon tetrachloride (5 to 40  $\mu$ l/kg) was administered intraperitoneally (IP) into mice in 0.2 ml of corn oil. In one group of mice, Thymol (50 to 300  $\mu$ l/kg) was given in corn oil immediately before the carbon tetrachloride injection. Control mice received either vehicle or Thymol only. Mice were killed and analyzed after 24 h.

The carbon tetrachloride damaged liver cells at a dose of 20  $\mu$ l/kg and was followed by a significant increase in serum alanine aminotransferase (ALT) activity and hepatic lipid peroxidation after 24 h. Histopathological examination confirmed hepatocellular necrosis. Oral administration of Thymol (300  $\mu$ l/kg) in a single dose resulted in significant attenuation of carbon tetrachloride-induced hepatotoxicity. The results of the study demonstrated that Thymol decreased carbon tetrachloride-induced liver injury in mice, which is evident by a significant decrease in serum ALT activity and lipid peroxidation (Alam et al. 1999).

Wong and McLean (1999) studied the activity of phenolic antioxidants (including *p*-Cresol) using in vitro DNA synthesis in male, Wistar rat liver, spleen, and testis as the end point. *p*-Cresol was tested at concentrations from 0.05 mM to ~0.8 mM. *p*-Cresol showed highly specific inhibition of DNA synthesis; in addition, there was very little protein synthesis inhibition observed. *p*-Cresol showed statistically significant differences between the percent of inhibition of DNA and protein synthesis in the rat testes at concentrations from ~0.08 mM (~10% inhibition) to ~0.8 mM (~80% inhibition).

### Penetration Enhancement

Kararli et al. (1995) reported on the in vitro effect of Thymol on the transdermal transport of azidothymidine (AZT) in vitro using skin from CD-1 mice and Sprague-Dawley rats. The vehicle was isopropyl alcohol (IPA)/water, propylene glycol (PG)/water, or IPA and PG/water; an occlusive patch was used. Thymol optimally increased the transport of AZT (by 33.8 times that of IPA/water formulation) at a 5% concentration.

These authors also studied the effect of Carvacrol on the in vitro transdermal transport of AZT in vitro using skin from CD-1 mice and Sprague-Dawley rats. In vivo transdermal bioavailability of AZT was determined in rats using Carvacrol enhanced gel

formulations. The vehicle was IPA/water, PG/water, or IPA and PG/water; an occlusive patch was used. At a concentration of 5%, Carvacrol optimally increased the transport of AZT (by 38.0 times that of IPA/water formulation) in mouse skin. At concentrations as low as 2% Carvacrol showed increased transport in mice. A 0.35-ml solution of Carvacrol (5%) reduced the transport of AZT significantly in dorsal and abdominal rat skin as compared to mouse skin; the transdermal flux values were 14.1, 21.7, and 31.9 mg/cm<sup>2</sup>/24 h in dorsal rat skin, abdominal rat skin, and mouse skin, respectively. The experiment was performed in vivo using three to five male Sprague-Dawley rats. The cumulative AZT flux rate was slightly decreased in vitro (0.8 mg/cm<sup>2</sup>/24 h) as compared to in vivo (0.91 mg/cm<sup>2</sup>/24 h), respectively (Kararli et al. 1995).

Arellano et al. (1996) studied the enhancing effect of Thymol on the percutaneous absorption of diclofenac sodium (DFS) using excised full-thickness abdominal skin from male Wistar rats. The vehicle was carbopol gel containing propylene glycol. Penetration time was up to 8 h and 1% Thymol had an enhancing effect on absorption.

Abreo et al. (1997) evaluated the effect of *p*-Cresol on the uptake and toxicity of aluminum in mouse hepatocytes (MH) in culture in a model system in which aluminum is bound to transferrin. *m*-Cresol and *o*-Cresol were also evaluated because of their structural similarity to *p*-Cresol.

All three Cresol isomers increased transferrin-associated net aluminum (Tf-Al) uptake in MH. Further studies were not performed on *m*-Cresol and *o*-Cresol because they are not typically detected in biological fluids. *p*-Cresol, when added to Tf-Al, increased MH Al uptake and resulted in significantly greater cell toxicity when assessed by cell growth and AST leakage into media compared with MH grown in Tf-Al alone. There was no increase in MH aluminum uptake or cell toxicity when *p*-Cresol was added to Al citrate. Time-response curves showed increased aluminum uptake and toxicity at *p*-Cresol concentrations of 3 mg/dl in culture media. Dose-response curves confirmed that aluminum uptake and cell toxicity were proportional to *p*-Cresol from 1.5 to 3 mg/dl (Abreo et al. 1997).

Kunta et al. (1997) evaluated the potential use of Carvacrol as a skin penetration enhancer (at concentrations of 1%, 5%, and 10%) in the transdermal delivery of propranolol hydrochloride (PL) using excised hairless mouse skin. In an in vitro permeation study Carvacrol had significantly better penetration than the other terpenes (menthol, limonene, and linalool) tested at concentrations of 5% and 10%. However, Carvacrol at 1% was not as effective as menthol for the first 8 h, but by 10 to 12 h Carvacrol was a more effective penetration enhancer. The authors attributed the skin transport enhancement of PL by Carvacrol to be a result of its hydrogen-binding ability through its hydroxyl group and its aromaticity.

Bhatia and Singh (1998) studied the effect of 5% terpenes (including Thymol) and iontophoresis on the in vitro permeability of leutinizing hormone-releasing hormone (LHRH) through the porcine epidermis. Five percent Thymol (in ethanol) was used

as a penetration enhancer for pretreatment. The donor concentration of LHRH used was  $3.92 \times 10^{-3}$  nmoles/ml. Pretreatment of the epidermis with 5% Thymol (in ethanol) increased the passive transport of LHRH as compared to control (no enhancer). Gao and Singh (1998) in a similar study observed that a 5% Thymol (in ethanol) solution significantly increased the permeability of tamoxifen through porcine epidermis. The partition coefficient of 5% Thymol in (ethanol) was  $1.65 \pm 0.01 \times 10^{-2}$ . In comparison, the partition coefficient of the control solution (50% ethanol) was  $1.35 \pm 0.01 \times 10^{-2}$ .

### Enzyme Effects

Gilbert et al. (1969) administered a single dose of Thymol or Carvacrol in arachis oil to albino CFE rats. Liver weight, processing-enzyme activities, hexobarbitone oxidase, and aminopyrene and UDPG dehydrogenase were measured 24 h after dosing. No effects on the activity of these enzymes were observed at 150 mg/kg, which was also reported as 1.5 mmol/kg.

Sternitzke et al. (1992) reported the effect of *m*-Cresol on hepatic microsomes using 7-ethoxycoumarin as a substrate for cytochrome P450. Male mice were given various pretreatments prior to the preparation of microsomes. Cytochrome P450 inhibition was observed at  $4 \times 10^{-3}$  to  $4 \times 10^{-4}$  M.

The ability of *m*-Cresol, *o*-Cresol, and *p*-Cresol to provoke binding spectra to cytochrome P450 or to affect the binding spectra performed by hexobarbital or metyrapone was also studied. There were no effects at doses from  $4 \times 10^{-3}$  to  $4 \times 10^{-4}$  M (Sternitzke et al. 1992).

### Effects on Muscle Contraction

Ito et al. (1974) studied the effect of Thymol on the electrical and mechanical properties of the guinea pig alimentary tract using the double gap method, strain gauge, and microelectrodes. The exposure concentration range was 0.5 to 1 mM and the vehicle was ethanol. In the stomach, Thymol (<0.5 mM) suppressed the generation of action potential and slow potential changes without any change in membrane potential and membrane resistance; increased concentrations of Thymol in the stomach did reduce membrane potential and membrane resistance. In the ileum and rectum, 1 mM Thymol suppressed spike generation, hyperpolarized the membrane, and decreased membrane resistance. The spontaneous mechanical activity of the smooth muscle cells of the stomach, ileum, and rectum were inhibited although a small contracture was induced in smooth muscle cells of the stomach.

Seeman et al. (1974) reported on the ability of calcium to reverse nerve-blocking action of Thymol using the phrenic nerve of the rat. The vehicle was a modified Locke's solution. The reversal of nerve blockage was produced by 0.002 and 0.00015 mol/L Thymol.

Takishima et al. (1979) studied the mechanism of the overshoot of calcium accumulation caused by Thymol in fragmented

sarcoplasmic reticulum prepared from rabbit white muscle. Influx and efflux of calcium were inhibited by Thymol which led to an initial increase of calcium followed by a marked decrease.

Van Den Broucke and Lemli (1980) stated that Thymol (in 0.4% aqueous ethanol) reduced contractions elicited by acetylcholine in guinea pig ileum; an  $ED_{50}$  of  $0.86 \times 10^{-4}$  M was calculated.

These authors also studied the ability of Carvacrol to reduce contractions elicited by acetylcholine in guinea pig ileum. The vehicle was 0.4% aqueous ethanol. Positive effects were observed, and an  $ED_{50}$  of  $1.0 \times 10^{-4}$  M was calculated (Van Den Broucke and Lemli 1980).

Viana et al. (1981) reported that Thymol or Carvacrol (0.2 to 2.0 mg) caused enhanced muscle contraction and contracture of isolated phrenic-diaphragm preparations and that 500  $\mu$ g Thymol or 500  $\mu$ g Carvacrol produced spasmogenic effects and inhibited contractions of the isolated rat uterus.

These authors also noted the effect of 100  $\mu$ g Thymol or 100  $\mu$ g Carvacrol on muscle tonus and motility following addition to rabbit duodenum in aerated Tyrode's solution. A spasmolytic effect decreasing both tonus and the amplitude of peristaltic movements was observed (Viana et al. 1981).

Van Den Broucke and Lemli (1982) reported on the reduction of contractions induced by 1-noradrenaline on the rat vas deferens. Thymol was administered at a concentration of  $1 \times 10^{-4}$  M. A reduction of contractions was observed and the authors concluded that Thymol is a calcium antagonist and it blocks nerve fiber conduction. Carvacrol was administered at a concentration of  $2 \times 10^{-4}$  M. Carvacrol reduced contractions induced by 1-noradrenaline on the rat vas deferens. The authors concluded that Carvacrol is a calcium antagonist and it blocks nerve fiber conduction.

These authors also studied the antagonistic effect of Thymol and Carvacrol on contractions induced in guinea pig ileum by carbachol, histamine, dimethyl phenyl piperazinium (DMPP), and  $BaCl_2$ . Isolated ileum was incubated with Thymol or Carvacrol for 5 min at concentrations between  $1 \times 10^{-4}$  M and  $2 \times 10^{-4}$  M. Thymol and Carvacrol each reduced ileum contractions (Van Den Broucke and Lemli 1982).

RIFM (2001e) reported on three studies. The first study evaluated the effect of Thymol on the force and rate of contractions in isolated spontaneously beating paired atria of guinea pigs. A decrease in force and rate of atrial contraction was seen as concentrations were increased from 10 to 30 to 100 and to 300 mg/L. Atropine pretreatment had no effect.

In the second study, the effect of Thymol on induced contractions of aorta isolated from New Zealand white rabbits was evaluated. Uncut ring and strip preparations were used. Relaxation of induced contractions was observed at concentrations of 10 to 300  $\mu$ g/ml.

The effect of Thymol on isolated rabbit intestines was examined in the third study. Thymol at concentrations from 0.001% to 0.01% lessened the tension in the intestines; the result was an effect on the muscles, not the nerves (RIFM 2001e).

### Blood Pressure

RIFM (2001e) reported on the effect of a single injection of Thymol on the heart rate and blood pressure of anesthetized Wistar rats. Rats were dosed with 1, 3, or 10 mg/kg Thymol. Blood pressure and heart rate decreased in a dose-dependent manner at 1 mg/kg Thymol and above. The effects were not altered by atropine, antihistamine, or  $\beta$ -adrenergic blockers.

The effect of Thymol on blood pressure and respiratory rate of rabbits was also studied. Thymol was injected into an ear vein in a vehicle of alcohol/propylene glycol/water. At a dose of 5 mg/kg, there was a transitory drop in blood pressure of 20 to 30 mm accompanied by respiration inhibition beginning about 5 to 10 min after dosing and lasting for 3 min (RIFM 2001e).

### Inflammation

Lorente et al. (1989) studied the potentiation of anti-inflammatory activity of a mixture of  $\alpha$ - and  $\beta$ -pinene (80 mg/kg each) by 1 mg/kg Thymol and 3 mg/kg Carvacrol using groups of eight female Wistar rats. The test mixture was administered in a 9:1 emulsion with Tween 80 1 h before injection with carageenan. The presence of Thymol and Carvacrol potentiated the anti-inflammatory activity of the mixture of  $\alpha$ - and  $\beta$ -pinene.

### Cellular Respiration

Hohenegger et al. (1988) investigated the effects of *m*-Cresol and *p*-Cresol on cellular respiration in male Sprague-Dawley rats (number not stated) in vitro and in vivo. In vitro respiration of rat diaphragm and liver slices was measured using the Warburg technique with air as the gas phase and the incubation medium of substrate free Krebs-phosphate buffer (pH 7.4).

Fasted (24-h) Sprague-Dawley rats, weighing between 150 and 300 g, were dosed intraperitoneally with a uremic toxin (*p*-Cresol or *p*-Cresol in mixture) dissolved in 0.9% sodium chloride solution (pH 7.0 to 7.4) at a volume of 0.5 to 1.0 ml/100 g body weight. Controls received solvent only. In part of the experiment, some rats were pretreated with 3 doses of 0.75 g/kg body weight triiodothyronine ( $T_3$ ) orally over 2 days before the fasting period began. Oxygen consumption was measured by a diaferometer or a digital respirometer between 1 and 6 h after injection.

In vitro, the following uremic toxins had no effect on cellular respiration of rat diaphragm and liver slices: *p*-Cresol, *p*-Cresol (with acetoin, methylguanidine, and putrescine), and *p*-Cresol (with methylguanidine) at a concentration of 30 mg/dl in the incubation medium. Six replicates were performed.

In vivo, *p*-Cresol at 200 mg/kg reduced oxygen consumption significantly from  $55.3 \pm 2.8$  in controls to  $43.7 \pm 3.0$  L/kg body weight/24 h in the treated group after a single ip dose (rats without  $T_3$  pretreatment). *p*-Cresol (50 mg/kg) and *m*-Cresol (100 and 200 mg/kg) had no effect on oxygen consumption (rats without  $T_3$  pretreatment). The experiment was not performed for *m*-Cresol and *p*-Cresol in rats with  $T_3$  pretreatment.

In  $T_3$  pretreated rats injected ip with 10 mg/kg *p*-Cresol (with methylguanidine, acetoin, and putrescine) or 10 mg/kg *p*-Cresol (with methylguanidine and putrescine), oxygen consumption decreased from  $75.4 \pm 10.5$  to  $60.1 \pm 5.5$  and  $75.4 \pm 5.5$  to  $59.7 \pm 7.7$  L/kg/24 h, respectively. In  $T_3$  pretreated rats injected ip with 10 mg/kg *m*-Cresol (with acetoin, putrescine, and methylguanidine) or 10 mg/kg *m*-Cresol (with methylguanidine), oxygen consumption decreased from  $65.6 \pm 3.8$  to  $51.4 \pm 5.4$  and  $64.0 \pm 5.5$  to  $46.2 \pm 2.4$  L/kg/24 h, respectively.

In rats without  $T_3$  pretreatment, dosed ip with 10 mg/kg *p*-Cresol (with methylguanidine, acetoin, and putrescine) or 10 mg/kg *p*-Cresol (with methylguanidine and putrescine), oxygen consumption decreased from  $68.4 \pm 7.1$  to  $50.6 \pm 7.1$  and  $68.4 \pm 7.1$  to  $60.4 \pm 2.9$  L/kg/24 h, respectively.

In rats without  $T_3$  pretreatment, dosed ip with 10 mg/kg *m*-Cresol (with acetoin, putrescine, and methylguanidine), *m*-Cresol (with putrescine and methylguanidine), or *m*-Cresol (with methylguanidine), oxygen consumption decreased from  $62.1 \pm 9.7$  to  $37.1 \pm 4.5$ ,  $53.0 \pm 2.3$  to  $45.7 \pm 2.2$ , and  $54.8 \pm 4.1$  to  $40.0 \pm 3.3$  L/kg/24 h, respectively. In rats pretreated with  $T_3$ , and dosed 10 mg/kg IP with *m*-Cresol (and putrescine), oxygen consumption was not significantly affected ( $69.4 \pm 2.2$  to  $60.8 \pm 9.9$  L/kg/24 h). The authors could not determine if uremic serum had an effect on tissue respiration of rat diaphragm or liver slices in vitro which contrasted with the in vivo data (Hohenegger et al. 1988).

### Estrogenic Activity

The specific binding ability of Chlorothymol to calf uterine estrogen receptor was evaluated. Chlorothymol was tested at concentrations up to 4.70 nM. The measured calf uterine estrogen receptor binding log  $IC_{50}$  (log nM) was 6.99 for Chlorothymol; in comparison,  $17\beta$ -estradiol (the positive control) had a log  $IC_{50}$  (log nM) value of 0.837. Both Chlorothymol and  $17\beta$ -estradiol exhibited measurable affinities to calf uterine estrogen receptors (Kramer and Giesy 1999).

## ANIMAL TOXICOLOGY

### Acute Oral Toxicity

**PCMC.** Five groups of male Wistar II rats were dosed with 1.0, 1.5, 2.0, 3.1, or 5.0 g/kg PCMC in (polyethylene glycol) by stomach tube (Bayer AG 1978). Intensified diuresis, sedation, disturbed respiration, trembling, and convulsions were observed. The oral  $LD_{50}$  of PCMC for Wistar II rats was 1830 mg/kg.

Robenek et al. (1980) gave groups of male Wistar rats a single oral dose of 400 mg/kg PCMC in peanut oil; controls were dosed with an equivalent amount of peanut oil only. All animals were killed 60 h after dosing and hepatic tissue was removed from the center of the right lobe of the liver for examination by electron microscopy.

After dosing, the animals' behavior changed; after 30 min, the animals were uneasy and had "ruffled-up" coats. These signs diminished after 1 h, but they were replaced by long "apathetic motions." After 24 h until study termination, the haircoats were again altered. At necropsy, the liver appeared slightly enlarged and was a pale red color with pale gray spots.

Light microscopy findings included a distinct dilation of the sinusoids with an activation of the Kupffer cells. The intercellular spaces were enlarged, and there were numerous cytoplasmic vacuoles. In electron micrographs, outpouchings of cell membranes were observed. A greater than normal number of lysosomes were around the bile canaliculi after dosing. Also, there was an increase in the number of mitochondria, many membrane-surrounded vacuoles, alterations in the intercellular space and in the rough endoplasmic reticulum, and an increase in the number and size of gap junctions. Additionally, the bile canaliculi were dilated and had irregularities and side branches which extended into the cytoplasm of adjacent hepatocytes (Robenek et al. 1980).

Five groups of Sprague-Dawley rats, 10 per sex per group, were dosed orally with PCMC in carbowax; males were dosed with 2000 to 7683 mg/kg and females with 1500 to 5762 mg/kg (Mobay Chemical Corporation 1981). Ataxia, wheezing, muscle fasciculations, tremors, convulsions, and salivation were observed in all dosed animals. The oral LD<sub>50</sub> of PCMC was 5129 mg/kg for male Sprague-Dawley rats and 3636 mg/kg for female Sprague-Dawley rats. These values are higher than reported for Wistar II rats earlier; but it was not clear if this is a strain or vehicle difference.

**Mixed Cresols.** Using 24 albino rats, the acute oral LD<sub>50</sub> for Mixed Cresols was determined to be between 1.0 and 2.0 g/kg of body weight. The probability that internal injury might result from incidental acute ingestion to industrial handling of Cresol was considered minimal by the researchers (Dow Chemical Company 1978).

**m-Cresol.** Uzhdavini et al. (1974) calculated the oral LD<sub>50</sub> of *m*-Cresol (in oil) to be 823 and 2010 mg/kg for mice and rats, respectively. The WHO (1995) reported oral LD<sub>50</sub> values of *m*-Cresol (10% in oil) of 600 mg/kg and 828 mg/kg for mice.

EI DuPont de Nemours & Co. (1983) assessed the acute oral toxicity of *m*-Cresol in male albino rats using 4 dose groups (147, 215, 316, and 464 mg/kg; five rats per group). Rats weighed between 174 and 186 g at the start of the experiment. The deaths occurred within 7 days of treatment; 0 of 5, 2 of 5, 4 of 5, and 5 of 5 rats died in the 147, 215, 316, and 464 mg/kg groups, respectively. Signs of intoxication were hypoactivity, tremors, convulsions, salivation, prostration, and death. There was inflammation of the gastrointestinal tract and hyperemia of liver, kidneys, and lungs in the rats that died. The LD<sub>50</sub> was 242 mg/kg.

Male albino rats fed a 10% aqueous solution of *m*-Cresol by gavage, had a reported LD<sub>50</sub> of 0.52 g/kg (Mellon Institute of Industrial Research 1949).

Deichmann and Witherup (1944) reported an LD<sub>50</sub> of *m*-Cresol (10% in oil) in rats by oral administration of 2020 mg/kg.

The WHO (1995) stated that the oral LD<sub>50</sub> of *m*-Cresol (10% in oil) in rats was 2010 mg/kg.

Deichmann and Witherup (1944) also administered *m*-Cresol (20% aqueous emulsions) orally to four rabbits (one per dose group) at doses from 620 to 2100 mg/kg. All doses of 1400 mg/kg and greater of *m*-Cresol were fatal to rabbits within 8 h.

**o-Cresol.** The *o*-Cresol (10% in oil) LD<sub>50</sub> was reported as 344 mg/kg in mice (WHO 1995). The oral LD<sub>50</sub> of *o*-Cresol (in oil) was calculated to be 344 and 1470 mg/kg when administered to mice and rats, respectively (Uzhdavini et al. 1974).

Hazelton Laboratories (1989d) reported acute toxicity in mice receiving *o*-Cresol. *o*-Cresol was administered to male ICR mice by gavage at a volume of 5 ml/kg and a dose of 50, 278, 525, 763, and 1000 mg/kg in trials 1 and 2 and 500, 750, 1000, 1250, 1500, and 1750 mg/kg in trial 3. Sterile deionized water was used as the vehicle for trial 1 and corn oil was used in trials 2 and 3. Animals that survived dosing were killed after 7 days. There were five male ICR mice (aged about 10 weeks) per dose group. Mice weighed from 26.2 to 38.4 g at predosing.

In trial 1, all animals dosed at 763 or 1000 mg/kg of *o*-Cresol began to tremble and three died within 10 min. One animal died in the 278 mg/kg group, four mice died in the 763 mg/kg dose group, and three mice died in the 1000 mg/kg dose group. All surviving animals had scruffy coats until the 6th day after dosing and had raspy breath until the 3rd day after dosing.

In trial 2, no mortalities occurred in any dose group exposed to *o*-Cresol. Within 5 min of dosing, animals exposed to *o*-Cresol at doses from 525 to 1000 mg/kg became prostrate. On day 4, mice exposed to 763 or 1000 mg/kg *o*-Cresol had slightly scruffy coats and made abnormal respiratory sounds that persisted throughout the study.

In trial 3, the doses were increased to a range of 500 mg/kg to 1750 mg/kg. No mice died in the 500 and 750 mg/kg dose groups. Two mice died in the 1000 mg/kg group, one mouse died in the 1250 mg/kg dose group, three mice died in the 1500 mg/kg dose group, and all mice died in the 1750 mg/kg dose group. Within 10 min of dosing all mice began to tremble and were languid. All animals exposed to 1000 mg/kg *o*-Cresol and greater had slightly rough coats and animals in the highest dose group were wheezing. By the 6th day all surviving animals appeared normal and healthy with the exception of one animal in the 1500 mg/kg dose group, which had squinty eyes (Hazelton Laboratories 1989d).

In a study reported by EI DuPont de Nemours & Co. (1983), the acute oral toxicity of *o*-Cresol in male albino rats was assessed using four dose groups (68, 100, 147, and 215 mg/kg; five rats per group). Rats weighed between 189 and 215 g at the start of the experiment. Deaths occurred within 4 h of treatment; 0 of 5, 2 of 5, 3 of 5, and 3 of 5 rats died in the 68, 100, 147, and 215 mg/kg groups, respectively. Signs of intoxication were hypoactivity, tremors, convulsions, salivation, prostration, and death. There was hemorrhage of the gastrointestinal tract and hyperemia of liver, kidneys, and lungs in the rats that died. The LD<sub>50</sub> was 121 mg/kg.

The Food and Drug Research Laboratories (FDRL) (FDRL 1975a) assessed the acute oral toxicity of *o*-Cresol in Wistar albino rats (200 to 300 g). Twenty-five rats were distributed into five dose groups (0.31, 0.625, 1.25, 2.5, and 5.0 g/kg) with three males and two females per group. Animals were fasted 24 h before dosing and food and water were available ad libitum after dosage. Animals were observed daily for 14 days following *o*-Cresol administration.

All animals treated with *o*-Cresol at doses of 1.25 g/kg and greater died on day 1. After 3 days there was 20% and 100% mortality in the 0.31 g/kg and 0.625 g/kg doses, respectively. The approximate acute oral LD<sub>50</sub> for *o*-Cresol was 0.36 g/kg. Gross examination revealed visceral hemorrhage (FDRL 1975a).

The LD<sub>50</sub> of *o*-Cresol (10% in oil) in rats was reported as 1350 mg/kg (Deichmann and Witherup 1944). The LD<sub>50</sub> of *o*-Cresol (10% in oil) in rats was reported as 1470 mg/kg (WHO 1995).

The *o*-Cresol (10% in oil) LD<sub>50</sub> was reported as 940 mg/kg in rabbits (WHO 1995). *o*-Cresol (20% aqueous emulsions) was administered orally to four rabbits (one per dose group) at doses from 420 to 1400 mg/kg. All doses of 940 mg/kg and greater of *o*-Cresol were fatal to rabbits within 4 h (Deichmann and Witherup 1944).

*p*-Cresol. The oral LD<sub>50</sub> of *p*-Cresol (in oil) was calculated to be 344 and 1460 mg/kg when administered to mice and rats, respectively (Uzhdavini et al. 1974).

Hazeltan Laboratories (1989f) reported acute toxicity in mice of *p*-Cresol. *p*-Cresol was administered to male ICR mice by gavage at a volume of 5 ml/kg and a dose of 100, 325, 550, 775, and 1000 mg/kg in two trials. Sterile deionized water was used as the vehicle for trial 1 and corn oil was used in trial 2. Animals that survived dosing were killed after 7 days. There were five male ICR mice (aged 10 weeks) per dose group.

In trial 1, all animals dosed at 775 and 1000 mg/kg of *p*-Cresol began to tremble and exhibit tonic convulsions. At 5 min, animals dosed with 550 mg/kg began to tremble and became lethargic. All animals in the 100 and 325 mg/kg dose groups survived, whereas animals dosed between 550 and 1000 mg/kg died. All surviving animals in the 325 mg/kg dose group had scruffy coats on the 2nd day after dosing, which persisted throughout the study. The mice in the 100 mg/kg dose group appeared healthy for the duration of the study.

In trial 2, no mortalities occurred in the 100, 325, and 550 mg/kg dose groups exposed to *p*-Cresol. One of five mice exposed to 775 mg/kg *p*-Cresol died and three of five mice exposed to 1000 mg/kg died. The mice in the 100 mg/kg group appeared healthy throughout the study. All mice at 325, 550, 775, and 1000 mg/kg were languid within 5 min of dosing, but most resumed normal activity after 10 min. Surviving mice in the 325, 550, 775, and 1000 mg/kg dose groups had scruffy coats through the first 6 days of the study and some remained languid or had hunched backs for part of the study (Hazeltan Laboratories 1989f).

Deer mice were fed white wheat seeds with 2% *p*-Cresol for 3 days and were observed for an additional 3 days. The calculated LD<sub>50</sub> was 1238 mg/kg. This figure was based on data from an associated repellency test (Schafer and Bowles 1985).

The *p*-Cresol (10% in oil) LD<sub>50</sub> values were reported as 440 mg/kg and 344 mg/kg in mice (WHO 1995).

The acute oral toxicity of *p*-Cresol in male albino rats was assessed using four dose groups (100, 147, 215, and 316 mg/kg; five rats per group). Deaths occurred within 12 h of treatment; 0 of 5, 0 of 5, 3 of 5, and 5 of 5 rats died in the 100, 147, 215, and 316 mg/kg groups, respectively. The signs of intoxication were hypoactivity, tremors, lacrimation, cyanosis, hemorrhagic rhinitis, convulsions, salivation, prostration, and death. There was hemorrhage of the gastrointestinal tract and hyperemia of liver, kidneys, and lungs in the rats that died. The LD<sub>50</sub> was 207 mg/kg (EI DuPont de Nemours & Co. 1983).

The LD<sub>50</sub> of *p*-Cresol (10% in oil) was reported as 1800 mg/kg in rats (Deichmann and Witherup 1944). The LD<sub>50</sub> of *p*-Cresol (10% in oil) was reported as 1430 mg/kg and 1460 mg/kg in rats (WHO 1995).

*p*-Cresol (20% aqueous emulsions) was administered orally to four rabbits (one per dose group) at doses from 280 to 1400 mg/kg. All doses of 620 mg/kg and greater of *p*-Cresol were fatal to rabbits within 12 h (Deichmann and Witherup 1944).

The WHO (1995) concluded that *o*-Cresol is the most toxic isomer, followed by *p*-Cresol and then *m*-Cresol. It appeared that the three isomers are more toxic to mice (three to four times) than rats by oral administration and that toxicity was dose-dependent. Cresols in oil were more toxic than Cresols in water. In rats and mice given *m*-Cresol, *o*-Cresol, or *p*-Cresol, clinical signs such as hypoactivity, lethargy, excess salivation, dyspnea, hemorrhagic rhinitis (*p*-Cresol only), uncoordination, prostration, muscle twitches and tremors, convulsions, and coma often preceded death. Necropsy revealed gastrointestinal inflammation and hemorrhage, as well as hyperemia of the lungs, liver, and kidney. No gross lesions were observed in rats treated with *o*-Cresol or *p*-Cresol and gastrointestinal tract inflammation was present in rats treated with *p*-Cresol.

*Thymol*. Livingston (1921) gave rabbits a single dose of undiluted Thymol in gelatin capsule. Survival time was noted. Five rabbits receiving 2 g/kg Thymol survived 1, 5, 11, 15, and 19 days. Two rabbits receiving 3 g/kg Thymol survived 4 and 8 days. One rabbit dosed 0.75 g/kg Thymol survived 9 days. Six rabbits receiving 0.5 g/kg Thymol survived 16, 18, 19, 24 (two animals), and 34 days. According to the author, the rabbits that died after 16 days did not likely die as a result of treatment.

In a similar experiment, rabbits were given a single dose of 50% Thymol (in olive oil) in gelatin capsules. Survival times were noted.

Rabbits receiving 0.25 g/kg Thymol survived 15 days (one animal) and 65+ days (four animals). Rabbits receiving 0.5 g/kg Thymol survived 18, 31, 34, 54, 65+ (five animals), and 120+ days (one animal). Rabbits receiving 0.75 g/kg Thymol survived 15, 20, and 65+ days (three animals). Rabbits receiving

1 g/kg Thymol survived 5, 6 (two animals), 37, 65+, and 76 days. Rabbits receiving 1.5 g/kg Thymol survived 6, 7, 64, and 120+ (two animals) days. Rabbits receiving 2 g/kg Thymol survived 2, 3, 4, 21, and 79 days. Rabbits receiving 3 g/kg Thymol survived 1 day. According to the author, the rabbits that died after 16 days did not likely die as a result of treatment (Livingston 1921).

McOmie et al. (1949) administered 620, 940, 1400, or 2100 mg/kg of Thymol (in cottonseed oil) by gavage to male mice. Mice were observed for 10 days. No mice administered 620 or 940 mg/kg died. The Thymol LD<sub>50</sub> was 1800 (±224) mg/kg. All deaths occurred within 24 h. Depression and prostration were noted at higher doses, only depression was noted at lower doses.

Jenner et al. (1964) dosed young, adult Osborne-Mendel rats by intubation with 20% Thymol in propylene glycol. Ten rats (5 male/5 female) were used in the study. The Thymol LD<sub>50</sub> in rats was 980 mg/kg (with 95% confidence limits of 817 to 1180 mg/kg). Rats died within 4 h to 5 days after dosing. Depression and ataxia were noted in most dose groups and coma at greater doses.

These authors also dosed guinea pigs by intubation with 20% Thymol in propylene glycol. Ten guinea pigs (5 male/5 female) were used in the study. The LD<sub>50</sub> in guinea pigs was 880 mg/kg (with 95% confidence limits of 740 to 1050 mg/kg) and guinea pigs died within 1 h to 10 days. Irritated gastrointestinal tract, tremors, coma, and respiratory failure were observed (Jenner et al. 1964).

The oral LD<sub>50</sub> of Thymol (in squalene) in ddY mice was 1200 mg/kg in males and 1050 mg/kg body weight in females. Acute toxic signs were hypoactivity and ataxic gait and there was some small intestinal congestion observed (Hasegawa et al. 1989).

The oral LD<sub>50</sub> values for Thymol in the mouse, cat, and rabbit were 640, 250, and 750 mg/kg respectively (Istituto Superiore di Sanità 1999).

RIFM (2001e) reported a study in which male mice were dosed by gavage with Thymol in an alcohol/propylene glycol/water vehicle. The LD<sub>50</sub> was calculated to be 640 mg/kg. Toxic signs included a decrease in spontaneous movements, piloerection, and paralysis of anterior limbs within 1 h. Death occurred within 5 to 6 h.

RIFM (2001e) also reported that groups of 8 white mice were dosed with a 10% Thymol solution in peanut oil by gavage. The observation period was at least 3 days. The following Thymol doses had no effect on mice: 0.5, 0.55, 0.6, 0.65, 0.7, 0.75, 0.8, 0.85, 0.9, 0.95, and 1.0 g/kg. Mice were administered Thymol at the following doses: 1.1 g/kg (1/8 mice died), 1.2 g/kg (3/8 mice died), 1.3 g/kg (4/8 mice died), 1.4 g/kg (6/8 mice died), and 1.5 g/kg (8/8 mice died).

In a final study reported by RIFM (2001e), groups of eight white mice were dosed with a 10% Thymol solution in aqueous emulsion by gavage. The following Thymol doses had no effect on mice: 0.5 and 0.55 g/kg. Mice were administered Thymol

at the following doses: 0.6 g/kg (1/8 mice died), 0.65 g/kg (4/4 mice died), 0.7 g/kg (6/8 mice died), 0.75 g/kg (8/8 mice died), and 0.8 g/kg (8/8 mice died).

*o*-Cymen-5-ol. In a study reported by CTFA (1980a), 10% *o*-Cymen-5-ol in an aqueous suspension of 0.5% carboxymethylcellulose was given by stomach tube to two groups of 4-week-old Slc-ddy mice. The sample of *o*-Cymen-5-ol used in the study had a purity of >99%. One group of 14 animals (7 females and 7 males) received the test suspension in a dose of 10 ml/kg, whereas a similar group of 14 mice was given a dose of 22 ml/kg. Animals were given food and water ad libitum prior to the single oral dose.

No change was noted in the "general condition" of the mice throughout the 7-day observation period. Females of both dosage groups and males administered 22 ml/kg showed lowered body weight gains the first day following dosing; however, weight gain was normal thereafter. Necropsy was performed 8 days after dosing and no lesions were found. No deaths were noted at either dosage level. The LD<sub>50</sub> of the test suspension was observed to be >22.0 ml/kg; the LD<sub>50</sub> of *o*-Cymen-5-ol was calculated as ~2.2 g/kg (CTFA 1980a).

*Carvacrol*. Livingston (1921) gave rabbits a single dose of undiluted Carvacrol in gelatin capsule. Survival time was noted. Six rabbits receiving 0.50 g/kg Carvacrol survived 8, 9, 10, 25, 32, and 35 days. One rabbit receiving 0.75 g/kg Carvacrol survived 2 days. Three rabbits receiving 1 g/kg Carvacrol survived 3, 18, and 20 days. Two rabbits receiving 1.5 g/kg Carvacrol survived 20 h and 2 days. Five rabbits receiving 2.0 g/kg survived for 18 h, 1, 3 (2 animals) and 4 days. One rabbit receiving 3.0 g/kg survived 6 days. One rabbit receiving 3.2 g/kg survived 3 days. However, the rabbits that died after 16 days did not likely die as a result of treatment.

In a similar experiment, rabbits were given a single dose of 50% Carvacrol (in olive oil) in gelatin capsules. Survival times were noted. In this study, five rabbits receiving 0.25 g/kg Carvacrol survived 65+ days (five animals). Nine rabbits receiving 0.5 g/kg Carvacrol survived 3 (two rabbits), 10, 40, and 65+ (five animals) days. Four rabbits receiving 0.75 g/kg Carvacrol survived 4, 27, and 65+ days (two animals). Five rabbits receiving 1 g/kg Carvacrol survived 2 (two animals), 3, 95, and 120+ days. Five rabbits receiving 1.5 g/kg Carvacrol survived 2, 3, 5, 16, and 120+ days. Five rabbits receiving 2 g/kg Carvacrol survived 18 h, 1 day (two animals), and 2 days (two animals). Two rabbits dosed 3 g/kg Carvacrol survived 1 day. However, the rabbits that died after 16 days did not likely die as a result of treatment. The study concluded that the toxicity of Carvacrol and Thymol is essentially the same in rabbits (Livingston 1921).

Ten (5 male/5 female) young, adult Osborne-Mendel rats were dosed with undiluted Carvacrol by intubation. The oral LD<sub>50</sub> was 810 mg/kg (with 95% confidence limits of 710 to 920 mg/kg) and rats died within 1 h to 3 days. Depression was noted within 10 min of dosing and coma within 1 h (Jenner et al. 1964).

The oral LD<sub>50</sub> for Carvacrol in rabbits was 100 mg/kg. No other information was available (Budavari 1989).

Rats were orally dosed with Carvacrol and observed for 14 days for mortality and systemic effects. There were 10 rats per dose group. Ten of 10 rats dosed with 5 g/kg died within 24 h and 3 of 10 rats dosed with 0.5 g/kg died. The oral LD<sub>50</sub> was determined to be >0.5 g/kg but <5.0 g/kg. No other details were available (RIFM 1976a as cited in RIFM 2001a).

### Acute Dermal Toxicity

*Mixed Cresols.* Vernot et al. (1977) reported that the dermal LD<sub>50</sub> of Mixed Cresols was 2000 mg/kg in rabbits.

Fiserova-Bergova et al. (1990) predicted the potential for the dermal toxicity of *m*-Cresol, *o*-Cresol, and *p*-Cresol using physical properties data, the threshold limit values (TLVs) (0.0220 mg/L) by inhalation, molecular weight (108.1 g), melting point (12°C, 31°C, 35°C), solubility in water (23.500, 25.000, and 24.000 mg/ml), and octanol-water partition coefficient (2.00, 2.00, 1.93) of *m*-Cresol, *o*-Cresol, and *p*-Cresol, respectively, were used. All three Cresol isomers were considered potential dermal toxicants.

*m*-Cresol. In a study reported by the Mellon Institute of Industrial Research (1949), the acute dermal toxicity of *m*-Cresol was investigated using rabbits. Undiluted *m*-Cresol applied to the clipped trunk of rabbits under an impervious sheeting resulted in an LD<sub>50</sub> of 1.80 ml/kg. Severe necrosis and erythema of the skin occurred. At 2.52 ml/kg tremors were noted from 1 to 4 h after the application. Most deaths occurred within the first 24 h. At necropsy, there was bloody urine in the urinary bladder, which can be indicative of kidney damage. *m*-Cresol was considered a moderate hazard by skin absorption because of its ability to cause skin necrosis and kidney damage.

The dermal LD<sub>50</sub> of *m*-Cresol was 1100 mg/kg in rats (Uzhdavini et al. 1974).

The dermal LD<sub>50</sub> of *m*-Cresol was 2830 mg/kg in rabbits after 24-h exposure (Vernot et al. 1977).

*o*-Cresol. The dermal LD<sub>50</sub> of *o*-Cresol was 620 mg/kg in rats (Uzhdavini et al. 1974).

FDRL (1975b) investigated the acute dermal toxicity of *o*-Cresol using rabbits. *o*-Cresol was applied to intact skin at the following doses: 0.02, 0.2, 0.431, 0.928, and 2.0 g/kg with five rabbits per dose group. The only deaths observed over the 14-day period were in the 2.0 g/kg group; one rabbit died on day 1 and another died on day 3. At gross examination there was renal hemorrhage. The acute dermal LD<sub>50</sub> was estimated to be greater than 2.0 g/kg.

The dermal LD<sub>50</sub> of *o*-Cresol was 890 mg/kg in rabbits after 24-h exposure (Vernot et al. 1977).

The acute dermal toxicity of *o*-Cresol was investigated using rabbits. The reported LD<sub>50</sub> was 890 mg/kg of body weight (Nishimura et al. 1994).

The dermal LD<sub>50</sub> of *o*-Cresol was reported to be 1.3 g/kg in rabbits. Two of two rabbits dosed with 5 g/kg *o*-Cresol died,

2/4 rabbits dosed with 2.5 g/kg died, 1/4 rabbits dosed with 1.25 g/kg died, 2/4 rabbits dosed with 0.625 g/kg died, and no effects were noted in rabbits dosed with 0.157 g/kg *o*-Cresol. Toxic signs were diarrhea and lethargy (RIFM 1980a as cited in RIFM 2001c).

Two of two guinea pigs dermally dosed with 5 g/kg *o*-Cresol died. No other details were available (RIFM 1980a as cited in RIFM 2001c).

*p*-Cresol. The dermal LD<sub>50</sub> of *p*-Cresol was 750 mg/kg in rats (Uzhdavini et al. 1974).

The dermal LD<sub>50</sub> of *p*-Cresol was 300 mg/kg in rabbits after 24-h exposure (Vernot et al. 1977).

The acute dermal toxicity of *p*-Cresol was investigated using rabbits. The reported LD<sub>50</sub> was 301 mg/kg of body weight (Nishimura et al. 1994).

The acute dermal LD<sub>50</sub> of *p*-Cresol in rabbits was 0.36 g/kg. There were six rabbits per dose group and the following doses were used: 0.05, 0.2, 0.3, 0.4, 0.5, 1, and 5 g/kg. There were no effects at the lowest dose. All rabbits died in the 1 and 5 g/kg dose groups. Four rabbits died in the 0.5 g/kg dose group. Three rabbits died in the 0.3 and 0.4 g/kg dose groups. One rabbit died in the 0.2 g/kg dose group. Toxic signs were dermal irritation, enteritis, and ataxia (RIFM 1973 as cited in RIFM 2001d).

*Thymol.* McOmie et al. (1949) reported that Thymol (in ether at a dose of 0.42 g/kg), applied to shaved rabbit back, made the skin turn parchment-like within 24 h. There was no apparent systemic effect. No deaths occurred. Complete necrosis of superficial skin layers occurred after 10 days.

Thymol was applied for 24 h to the clipped abraded abdominal skin of 10 New Zealand rabbits (weighing 1.9 to 2.4 kg). Observations were made for mortality and toxic effects for 7 days, which was followed by gross necropsy. There were no deaths at the 2 g/kg dose. At 24 h, rabbits dosed 2 g/kg had moderate erythema (10 of 10 rabbits), moderate edema (8 of 10 rabbits), or slight edema (2 of 10 rabbits). On day 7, moderate erythema was observed in 3 of 10 animals and slight erythema was noted in 4 of 10 animals. Necropsy was performed on all animals, but the results were not provided (RIFM 1972b as cited in RIFM 2001e).

*Carvacrol.* McOmie et al. (1949) reported a study in which Carvacrol was placed in rubber cuffs and applied to the skin of rabbits for 6 h. Carvacrol (2.7 cc/kg) caused immediate irritation and the skin became erythematous. A leather-like appearance was evident within 4 h. Complete necrosis and eventual sloughing and scar formation were present before death at 72 h post exposure.

Carvacrol was applied to the skin of rabbits; mortality and systemic effects were observed over 14 days. There were 10 rabbits per dose group. Nine of 10 rabbits dosed with 5 g/kg Carvacrol died. No other details were available (RIFM 1976a as cited in RIFM 2001a).

Carvacrol was applied to the skin of ten rabbits at a dose of 5 g/kg. Three of 10 rabbits died. The LD<sub>50</sub> was >5 g/kg (RIFM 1977 as cited in RIFM 2001a).

Carvacrol was applied to the skin of 10 rabbits at a dose of 2.5 or 5 g/kg. No rabbits in the low-dose group died (0/2) and one out of eight rabbits in the 5 g/kg group died. The rabbit that died in the 5 g/kg group had a bluish tinge to eyes, nose, and ears and slight lethargy was also noted. Panting was observed in one other rabbit. The acute dermal LD<sub>50</sub> was >5 g/kg and necropsy findings were routine (RIFM 1977 as cited in RIFM 2001a).

### Acute Inhalation Toxicity

**PCMC.** Upon a 4-h exposure, the inhalation LC<sub>50</sub> for rats was >583 mg PCMC-NA/m<sup>3</sup> air (Paulus and Genth 1983).

**Mixed Cresols.** The exposure of four albino rabbits to a saturated atmosphere of Mixed Cresols with a concentration of 22 mg/l for 6 min at room temperature resulted in 100% mortality. Severe eye and nasal irritation was observed in all animals before death (Dow Chemical Company 1978).

According to the WHO, acute poisoning with Cresol vapor is unlikely due to the low vapor pressure of these compounds. Inhalation of an aerosol and vapor mixture, however, may cause death (WHO 1995).

**m-Cresol.** The Mellon Institute of Industrial Research (1949) investigated the acute inhalation toxicity of *m*-Cresol using rats. Six rats were exposed to a saturated solution of *m*-Cresol for 8 h by bubbling air at 2.5 L/min with 50 ml of *m*-Cresol. In a separate experiment, six rats were exposed to a mist drawing *m*-Cresol from a 170°C bath. Observations were made for 14 days following exposure. All rats exposed to the saturated *m*-Cresol solution gained weight, whereas only one rat exposed to the *m*-Cresol mist did not gain weight. Inhalation of *m*-Cresol by vapor or mist was considered a slight hazard for a limited single exposure.

The LC<sub>50</sub> of *m*-Cresol was reported as 58 mg/m<sup>3</sup> in rats (WHO 1995).

**o-Cresol.** The FDRL (1975e) investigated the acute inhalation toxicity of *o*-Cresol using albino rats. Thirty albino rats (FDRL/Wistar stock) weighing between 200 and 300 g were distributed into three groups of five males and five females per group. The atomizer used delivered a very fine mist but the particle size was not stated. Rats were exposed at either 0.2, 2.0, or 20.0 mg/L of aerosolized *o*-Cresol for 6 h. Observations were made daily for 14 days after the exposure. No rats died in the study, but eye irritation was observed during the exposure period and these effects cleared within 24 h. The acute inhalation LC<sub>50</sub> was estimated to be greater than 20 mg/L when *o*-Cresol is aerosolized from an alcohol solution.

Cats exposed to 5 to 9 mg/m<sup>3</sup> of *o*-Cresol were reported to have mucosal irritation, as indicated by parotid gland secretions (U.S. Department of Health and Human Services 1992).

In mice, the LC<sub>50</sub> of the vapor/aerosol mixture of *o*-Cresol was 178 mg/m<sup>3</sup> (exposure duration not specified). Clinical signs of toxicity included irritation of mucous membranes and neuromuscular excitation that progressed from twitching to con-

vulsions. Hematuria was evident at very high concentrations. Microscopically there were changes in the liver, such as fatty degeneration and centrilobular necrosis, and in the kidneys there was edema, swelling of the glomeruli, degeneration of the tubular epithelium and perivascular hemorrhage (WHO 1995). The median LC<sub>50</sub> concentration of *o*-Cresol and *p*-Cresol was reported as 29 mg/m<sup>3</sup> in rats (WHO 1995).

**Thymol.** The sedative effects of air containing Thymol (1.5 ml) on four female Swiss mice for one hour were evaluated. Total test material volume was 20 to 50 mg. Blood samples were taken at 0, 30, 60, and 90 min of inhalation exposure. The effect with and without caffeine was evaluated using a 0.5-ml ip injection of a 0.1% caffeine solution. Motility was significantly increased in mice treated with Thymol only (+33.02%) and with Thymol/caffeine (+19.05%) as compared to controls. Thymol was not detected in the blood after 90 min of exposure, the detection limit was  $3.40 \times 10^{-1}$  ppm (Buchbauer et al. 1993).

### Acute Parental Toxicity

**PCMC.** Wein (1939) reported a study in which five groups of 10 or 20 mice (sex not specified) were dosed subcutaneously with 100 to 500 mg/kg PCMC as a 0.4% aqueous solution. Toxic signs appeared within 5 min and included severe muscular tremors, especially in the fore and hind limbs. Death from respiratory failure usually occurred within 3 h, with some animals dying within 30 min. The calculated subcutaneous LD<sub>50</sub> of PCMC for mice was 360 mg/kg.

Three groups of 10 albino rats per group (sex not specified) were dosed by subcutaneous injection with a 0.4% aqueous solution of 30, 400, or 500 mg/kg PCMC. Observations included tremors followed by death in approximately 15 min. The calculated subcutaneous LD<sub>50</sub> was 400 mg/kg PCMC.

Five groups of 10 or 20 mice (sex not specified) were dosed intravenously with 100 to 500 mg/kg PCMC as a 0.4% aqueous solution. The calculated intravenous LD<sub>50</sub> of PCMC for mice was 70 mg/kg (Wein 1939).

The Robenek et al. (1980) acute toxicity study described in Acute Oral Toxicity also included testing in which a single subcutaneous dose of 400 mg/kg PCMC in peanut oil was administered to rats; the same procedures were followed. The same general behavioral and macroscopic hepatic changes and light microscopy findings were observed. In electron microscopic examination of the liver, the reported findings included enlarged sinusoids, an increase in detritus in the Kupffer cells, enlarged intercellular spaces, and an increased number of cytoplasmic vacuoles. There was also an increase in the number of lysosomes, with some of these lysosomes being comparable in size to mitochondria. The rough endoplasmic reticulum appeared disorganized, often surrounded by mitochondria and with an increase in the loss of ribosomes. Additionally, there was an increase in mitochondria, which often appeared pleomorphic; some of the mitochondria had a long shape and showed a tear

in the outer membrane. Also, a translucent matrix accompanied by a decrease in mitochondrial cristae was observed.

The percutaneous LD<sub>50</sub> of PCMC for rats was >500 mg/kg (Paulus and Genth 1983).

*m-Cresol.* Deichmann and Witherup (1944) injected *m*-Cresol (0.5% in aqueous solutions) intravenously into four rabbits (one per dose group) at doses from 120 to 420 mg/kg. All doses of 280 mg/kg and greater of *m*-Cresol were fatal to rabbits within 15 h.

These authors also injected *m*-Cresol (10% in olive oil) subcutaneously into seven cats (one per dose group) at doses from 80 to 940 mg/kg. All doses of 180 mg/kg and greater of *m*-Cresol were fatal to cats within 27 h (Deichmann and Witherup 1944).

Matsumoto et al. (1963) injected *m*-Cresol (in water) subcutaneously into rats. A single injection (10 cc/100 g body weight) of 0.1 M *m*-Cresol caused convulsions within 4 to 7 min. There were 8 to 12 convulsions for the next 30 min.

Angel and Rogers (1972) injected *m*-Cresol (in saline) intraperitoneally in mice. *m*-Cresol induced convulsions in 50% of mice at 0.94 mM/kg. Confidence limits of 95% were between 0.71 and 1.26.

A single dose of *m*-Cresol (200 mg/kg) was injected intraperitoneally in male DBA/2Ncr/BR mice and caused lethargy, piloerection, and lacrimation within 21.5 h. The animals were killed after 21.5 h and were used for a sister-chromatid exchange (SCE) assay (Cheng and Kligerman 1984).

A single dose of *m*-Cresol was injected intraperitoneally in mice to determine an LD<sub>50</sub>. *m*-Cresol was isolated from a smoke condensate fraction and administered in isotonic saline solution to give a dose of 0.1 ml/10 g body weight. The LD<sub>50</sub> of *m*-Cresol was 450 mg/kg body weight (Sternitzke et al. 1992).

*o-Cresol.* Deichmann and Witherup (1944) injected *o*-Cresol (10% in olive oil) subcutaneously into nine cats (one per dose group) at doses from 24 to 940 mg/kg. All doses of 55 mg/kg and greater were fatal to cats within 60 h.

These authors also injected *o*-Cresol (0.5% in aqueous solutions) intravenously into four rabbits (one per dose group) at doses from 80 to 280 mg/kg. All doses of 180 mg/kg and greater were fatal to rabbits within 10 h (Deichmann and Witherup 1944).

Matsumoto et al. (1963) injected *o*-Cresol (in water) subcutaneously into rats. A single injection (10 cc/100 g body weight) of 0.1 M *o*-Cresol caused convulsions within 4 to 7 min. There were 7 to 10 convulsions for the next 30 min.

Angel and Rogers (1972) injected *o*-Cresol (in saline) intraperitoneally in mice. *o*-Cresol induced convulsions in 50% of mice at 1.08 mmole/kg. Confidence limits of 95% were between 0.91 and 1.28 mmole/kg.

Groups of five albino mice were dosed with *o*-Cresol (in DMSO) intraperitoneally. Mice were observed for 24 h after dosing. The LD<sub>50</sub> was calculated to be 2.3 mmole/kg (Biagi 1975).

A single dose of *o*-Cresol (200 mg/kg) was injected intraperitoneally into male DBA/2Ncr/BR mice which caused lethargy,

piloerection, and lacrimation within 21.5 h. The animals were killed after 21.5 h and were used for a SCE assay (Cheng and Kligerman 1984).

A single dose of *o*-Cresol was injected intraperitoneally in mice to determine an LD<sub>50</sub>. *o*-Cresol was isolated from a smoke condensate fraction and administered in an isotonic saline solution to give a volume of 0.1 ml/10 g body weight. The LD<sub>50</sub> of *o*-Cresol was 350 mg/kg body weight (Sternitzke et al. 1992).

*p-Cresol.* Deichmann and Witherup (1944) injected *p*-Cresol (10% in olive oil) subcutaneously into nine cats (one per dose group) at doses from 36 to 940 mg/kg. All doses of 80 mg/kg and greater were fatal to cats within 120 h.

These authors also injected *p*-Cresol (0.5% in aqueous solutions) intravenously into four rabbits (one per dose group) at doses from 80 to 280 mg/kg. All doses of 180 mg/kg and greater were fatal to rabbits within 15 h (Deichmann and Witherup 1944).

*p*-Cresol (in water) was injected subcutaneously into rats. A single injection (10 cc/100 g body weight) of 0.1 M *p*-Cresol caused convulsions within 4 to 7 min. There were 20 to 30 convulsions for the next 35 min (Matsumoto et al. 1963).

*p*-Cresol (in saline) was injected intraperitoneally in mice. *p*-Cresol induced convulsions in 50% of mice at 1.02 mM/kg. Confidence limits of 95% were between 0.68 and 1.54 mM/kg (Angel and Rogers 1972).

A single dose of *p*-Cresol (75 mg/kg) injected intraperitoneally in male DBA/2Ncr/BR mice caused lethargy, piloerection, and lacrimation within 21.5 h. The animals were killed after 21.5 h and were used for a SCE assay (Cheng and Kligerman 1984).

A single dose of *p*-Cresol was injected intraperitoneally in mice. *p*-Cresol was isolated from a smoke condensate fraction and administered in an isotonic saline solution to give a volume of 0.1 ml/10 g body weight. The LD<sub>50</sub> of *p*-Cresol was 150 mg/kg body weight (Sternitzke et al. 1992).

*Thymol.* Möller (1939) dosed 4 young male guinea pigs subcutaneously once a day with Thymol (in olive oil) for 8 to 9 days to assess the effect of Thymol on the thyroid gland. The dose was 20 or 40 mg/day (106 or 233 mg/kg/day). Oxygen consumption was monitored and a histological examination of the thyroid was done. Clear thyroid activation was seen in 2 animals and weakly in a third animal. There was no effect on oxygen consumption in any animal.

A similar experiment was performed where Thymol (in olive oil) was injected nine times over 16 days into four young male guinea pigs. There was no effect on oxygen consumption. There was thyroid activation in the 60 mg (243 mg/kg/day) dose after two injections, 80 mg (313 mg/kg/day) dose after three injections, and 100 mg (375 mg/kg/day) dose after four injections (Möller 1939).

Caujolle and Franck (1944) perfused 6 dogs with Thymol for 20 to 25 min. Effects on blood pressure, respiration, and survival were monitored. The vehicle was oil. At a dose of 0.06 g/kg

clinical signs included convulsions and respiratory arrest. The LD<sub>50</sub> of Thymol was 0.15 g/kg.

Matsumoto et al. (1963) injected Thymol (in ethylene glycol) subcutaneously into rats. A single injection of Thymol (1.0 to 3.0 cc/100 g body weight) did not cause convulsions. No additional information was available.

Groups of male and female A/He5 mice received six injections of Thymol over 2 weeks. Mice weighed 18 to 30 g and were six to eight weeks old. Serial two-fold dilutions of Thymol were administered. The maximum tolerated dose (MTD) of all five mice was 0.25 g/kg (Stoner et al. 1973).

In five male SPF mice administered six to eight doses of Thymol intravenously, the LD<sub>50</sub> was calculated as 100 mg/kg (James and Glen 1980).

Thymol was injected intraperitoneally into Swiss mice at doses from 33.3 to 233.3 mg/kg (three mice per test group). Mice were observed for up to 3 days after Thymol administration. Mice dosed at 33.3 mg/kg showed no effects. Mice dosed at 50 mg/kg had nonspecific effects and slight ataxia, but no mice died. One out of three mice dosed at 73.3 mg/kg died; ataxia and decreased spontaneous motor activity were noted. All mice dosed at 110, 166.6, and 233.3 mg/kg died. Ataxia, decreased spontaneous motor activity, and somnolence were noted at doses from 110 to 233.3 mg/kg. The LD<sub>50</sub> in mice was calculated to be 110 mg/kg, and actual testing at this dose resulted in the death of three of three mice (Viana et al. 1981).

The subcutaneous LD<sub>50</sub> values of Thymol were reported as 800 and 1600 mg/kg in the mouse and rat, respectively; and the intravenous LD<sub>50</sub> values for Thymol in the rabbit, mouse, and dog were reported as 60, 100, and 150 mg/kg, respectively (Istituto Superiore di Sanità 1999).

In male mice dosed subcutaneously with Thymol in an alcohol/propylene glycol/water vehicle, the LD<sub>50</sub> was calculated to be 243 mg/kg. No other information was available (RIFM 2001e).

*o*-Cymen-5-ol. Six animals per group were used to determine the ip LD<sub>50</sub> of *o*-Cymen-5-ol for mice (sex and strain not specified) (Osaka Municipal Hygienic Laboratory 1953). Animals from each group were dosed with 0.3, 0.4, 0.5, or 0.6 g/kg *o*-Cymen-5-ol in an oil-in-water emulsion. The LD<sub>50</sub> for mice was 0.47 g/kg.

*Carvacrol*. Six dogs were perfused intravenously in the femoral vein with Carvacrol for 20 to 25 min. Effects on blood pressure, respiration, and survival were monitored. The lethal dose of Carvacrol was 0.31 g/kg (Caujolle and Franck 1944).

Viana et al. (1981) injected Carvacrol intraperitoneally into Swiss mice at doses from 33.3 to 233.3 mg/kg (3 mice per test group). Mice were observed for up to 3 days after Carvacrol administration. Mice receiving 33.3 mg/kg Carvacrol showed no effects. Mice dosed with 50 mg/kg Carvacrol had nonspecific effects and slight ataxia but no mice died. All mice dosed at 110, 166.6, and 233.3 mg/kg Carvacrol died. Ataxia, decreased spontaneous motor activity, and somnolence were noted at doses

from 110 to 233.33 mg/kg. The Carvacrol LD<sub>50</sub> in mice was calculated to be 73.3 mg/kg, and actual testing at this dose resulted in the death of two of three mice. Ataxia and decreased spontaneous motor activity were noted.

RIFM (2001a) reported a study in which guinea pigs were injected intraperitoneally with Carvacrol in oil. No further experimental details were provided. At a 10% concentration, damage to the gastrointestinal tract and death by emaciation were noted a few days after dosing. Intense intraperitoneal reaction with multiple adhesions was produced.

Guinea pigs also were injected subcutaneously four times at 5-day intervals and twice at 10-day intervals with Carvacrol in oil. No further experimental details were provided. At 1% concentration, results stated as “perfectly supportable”, which was assumed to mean no adverse effects (RIFM 2001a).

### Short-Term Oral Toxicity

*PCMC*. Madsen et al. (1986) dosed groups of 20 Wistar SPF rats, 10 males and 10 females per group, with 50, 200, or 400 mg/kg/day PCMC in food-grade soybean oil by gavage at a volume of 5 ml/kg/day for 28 days. A control group was dosed with soybean oil only. The animals were examined twice daily, and body weights and feed consumption were determined weekly. Blood samples were taken from eight males and eight females in each group for hematological and clinical chemistry analyses after 21 days of dosing. All rats were necropsied at study termination. The only toxicological sign observed was a statistically significant decrease in body weight gains for the males and females dosed with 400 mg/kg PCMC. Relative organ weights were comparable for all groups, and the hematological and clinical chemistry parameters were normal. No dose-related pathological changes were observed. The no observed effect level (NOEL) was 200 mg/kg/day PCMC.

Rats were fed 2500, 5000, or 10,000 ppm PCMC and controls were fed untreated feed (number per group, sex, and dose duration not stated) (Bayer AG 1992). Male rats of the 10,000 ppm dose group had reduced body weight gains. Dose-related effects were not observed for males of the 2500 and 5000 ppm dose groups or for any of the female rats.

*m-Cresol, o-Cresol, and p-Cresol (NTP Study)*. In 28-day studies conducted by the National Toxicology Program (NTP) (1992a), the toxicity of *o*-Cresol, *m*-Cresol, *p*-Cresol, or *m*-Cresol/*p*-Cresol mixture (60:40) in the diet was evaluated in F344/N rats (4 to 5 weeks old) and B6C3F1 mice (4 weeks old). Groups of five animals of each species and sex were fed diets containing 0, 300, 1000, 3000, 10,000, or 30,000 ppm. Feed and water was available ad libitum and supplied twice weekly. Feed consumption was recorded twice weekly, animals were observed for signs of toxicity twice daily, and weight was recorded.

*m-Cresol arm of NTP study—mice*. Two male and two female mice receiving 30,000 ppm *m*-Cresol, one female receiving 10,000 ppm *m*-Cresol, and one control mouse died (or were killed moribund).

Mean final body weights were significantly decreased in high-dose male and female mice, and mean body weight gains in high-dose males were also decreased. During the first week of the study, feed consumption was decreased in high-dose males and females, and decreased feed consumption was observed in week three for females. Clinical signs of toxicity such as hunched posture, rough hair coat, and thin appearance were observed in high-dose mice given *m*-Cresol. Tremors and lethargy were exhibited in high-dose mice and hypothermia was noted in high-dose females. Hunched posture and rough hair coat were also observed in mice dosed with 10,000 ppm *m*-Cresol. Female mice receiving 10,000 ppm *m*-Cresol showed labored respiration, lethargy, and sunken eyes.

At study termination, relative liver weights were increased in all female dose groups and the three highest male dose groups as compared to controls. Increased relative kidney weights were observed in the female high-dose group and males from the 3000 ppm dose group. High-dose males had significantly increased relative brain weight. No gross lesions were noted at necropsy. Histopathologically there was mammary gland, ovarian, and uterine atrophy in the three females that survived in the high-dose group.

*m*-Cresol arm of NTP study—rats. All rats given *m*-Cresol survived until the end of the study.

Decreases in mean final body weights and mean body weight gains in rats given 30,000 ppm *m*-Cresol were statistically significant as compared to controls. During the first week of the study, feed consumption was decreased in high-dose males and females by as much as 47% and 38%, respectively. No clinical signs of toxicity were observed in rats given *m*-Cresol.

At study termination, relative liver weights were significantly increased in the rats given 10,000 or 30,000 ppm *m*-Cresol. Males receiving 10,000 ppm also had increased liver weight as compared to controls. Relative brain weight and relative kidney weight were marginally increased in the high-dose group. No gross lesions were noted at necropsy; histopathologically there was minimal to mild uterine atrophy in four of five females in the high-dose group.

*o*-Cresol arm of NTP study—mice. Two male mice and one female mouse receiving 30,000 ppm *o*-Cresol died (or were killed moribund) between days 5 and 9 of the study.

Mean final body weights were significantly decreased in high-dose mice and mean body weight gains in the two highest dose groups were also decreased. During the first week of the study, feed consumption was decreased in high-dose males and females, and for the first 3 days of the study feed consumption was decreased in males receiving 3,000 and 10,000 ppm *o*-Cresol. Clinical signs of toxicity such as hunched posture, rough hair coat, and thin appearance were observed in high-dose mice given *o*-Cresol. Tremors and lethargy were exhibited in high-dose mice and hypothermia, rapid breathing, and tremors were noted in high-dose males.

At study termination, relative liver weights were increased significantly in the three highest dose groups as compared to controls. Relative kidney weights were increased for females receiving 10,000 and 30,000 ppm *o*-Cresol, and males receiving 10,000 ppm *o*-Cresol. High-dose females had significantly increased relative brain weight. No gross lesions were noted at necropsy. Histopathologically there was uterine atrophy at the two highest doses and ovarian atrophy at the highest dose.

*o*-Cresol arm of NTP study—rats. All rats given *o*-Cresol survived until the end of the study.

A decrease in mean final body weights were statistically significant in female rats given 30,000 ppm *o*-Cresol as compared to controls. Mean body weight gains in rats given 30,000 ppm *o*-Cresol were also significantly decreased as compared to controls. During the first week of the study, feed consumption was decreased in high-dose males and females by as much as 58% and 53%, respectively. No clinical signs of toxicity were observed in rats given *o*-Cresol.

At study termination, liver weights were significantly increased in the rats given 10,000 or 30,000 ppm *o*-Cresol and relative liver weights were significantly increased in the three highest dose groups for males and the two highest dose groups for females. Relative brain weights were slightly increased in high-dose females and relative kidney weights were significantly increased in rats of both sexes from the two highest dose groups. No gross lesions were noted at necropsy and no treatment-related lesions were noted microscopically.

*p*-Cresol arm of NTP study—mice. All high-dose male and female mice and one male receiving 10,000 ppm *p*-Cresol died (or were killed moribund).

Mean final body weights and mean body weight gains were significantly decreased in surviving male mice receiving 10,000 ppm *p*-Cresol as compared to controls. During the first 2 weeks of the study, feed consumption was decreased in females receiving 10,000 ppm *p*-Cresol. Feed consumption was decreased in males receiving 10,000 ppm for the first 5 days and at the beginning of week 2. High-dose animals that died had one or more of the following clinical signs of toxicity: hunched posture, lethargy, rough hair coat, hypothermia, labored respiration, and thin appearance. Males receiving 10,000 ppm displayed hunched posture, hypothermia, labored respiration, lethargy, paleness, rough hair coat, and thin appearance.

At study termination, relative liver weights were increased significantly in males receiving 10,000 ppm and females receiving 3000 or 10,000 ppm *p*-Cresol as compared to controls. Relative kidney weights were increased for males receiving 3,000 and 10,000 ppm *p*-Cresol. Males receiving 3,000 ppm *p*-Cresol also had increased relative heart weights. High-dose females had significantly increased relative brain weights.

No gross lesions were noted at necropsy. Histopathological examination of the 10,000 ppm group revealed the nose as the target organ in males and females. Minimal to mild hyperplasia

of the nasal respiratory epithelium was present in all animals in this dose group. The NOEL for this lesion was 300 ppm in males and a NOEL was not found for females. The high-dose mice had additional lesions that did not occur at lower doses, such as lymphoid necrosis and depletion in various lymphoid tissues, which were considered secondary to moribund condition or stress. Renal and hepatic necrosis and bone marrow hypocellularity were possibly a direct result of Cresol toxicity in high-dose mice.

*p-Cresol arm of NTP study—rats.* All rats given *p*-Cresol survived until the end of the study.

Decreases in mean final body weights and mean body weight gains in rats given 30,000 ppm *p*-Cresol were statistically significant as compared to controls. During the first week of the study, feed consumption was decreased in high-dose males and females by as much as 75% and 79%, respectively. During the first week, clinical signs of toxicity such as hunched posture, rough hair coat, and thin appearance were observed in high-dose rats given *p*-Cresol.

At study termination, relative liver weights were significantly increased in the male rats given 10,000 or 30,000 ppm *p*-Cresol and female rats given 3000, 10,000, and 30,000 ppm *p*-Cresol. There were significant increases in relative kidney weights in the two highest dose groups for males and the female high-dose groups. Relative brain weights were slightly increased in high-dose rats. High-dose males had significantly increased testis weights. The changes in relative brain and testis weights were considered a result of decreased body weight gains. No gross lesions were noted at necropsy. Bone marrow hypoplasia and uterus, ovary, and occasional mammary gland atrophy were seen primarily at the highest doses, but also at 10,000 ppm *p*-Cresol. Atrophy and regenerative changes in the nasal epithelia and forestomach were noted; this was presumably a direct result of the irritant effects of the chemical or its vapor.

*m-Cresol/p-Cresol arm of NTP study—mice.* All mice receiving *m*-Cresol/*p*-Cresol survived to the end of the study.

Mean final body weights and mean body weight gains were significantly decreased in high-dose mice as compared to controls. Mean gains were also depressed in the 3000 ppm and 10,000 ppm male dose groups. During the first week of the study, feed consumption was decreased in males and females receiving 30,000 ppm *m*-Cresol/*p*-Cresol. Feed consumption was decreased in high-dose females during the third week. Clinical signs of toxicity, observed in high-dose mice, included alopecia, dehydration, hunched posture, hypothermia, lethargy, rough hair coat, and thin appearance.

At study termination, relative liver weights were increased significantly in males receiving 1000 ppm and higher, and in females receiving 3000 ppm and higher. High-dose males had significantly increased relative brain weights and relative testis weights. High-dose females had significantly decreased brain weights and increased relative kidney weights and relative brain weights. No gross lesions were noted at necropsy. Microscopi-

cally, there was respiratory epithelial hyperplasia. In high-dose mice, olfactory atrophy and respiratory metaplasia were noted. Also, all high-dose mice had minimal to mild bronchiolar epithelial hyperplasia. Bone marrow hypocellularity occurred in two of five males and one of five females at the high dose. Uterine and ovarian atrophy were noted in one high-dose female. Minimal esophageal and forestomach epithelial hyperplasia were noted in one high-dose male.

*m-Cresol/p-Cresol arm of NTP study—rats.* All rats given *m*-Cresol/*p*-Cresol survived until the end of the study.

Mean final body weights were significantly lower in male rats in the high-dose group. Decreases in mean final body weight gains in rats given 30,000 ppm *m*-Cresol/*p*-Cresol were statistically significant as compared to controls. During the first week of the study, feed consumption was decreased in high-dose males and females by as much as 76% and 73%, respectively. During the first week, a thin appearance was observed in all high-dose rats given *m*-Cresol/*p*-Cresol.

At study termination, relative kidney weights were significantly increased in male and female rats given 10,000 or 30,000 ppm *m*-Cresol/*p*-Cresol. There were significant increases in relative liver weights in the three highest dose groups for males and the four highest dose groups for females. High-dose males had significantly increased relative brain weights and relative testis weights. The changes in relative brain and testis weights were considered a result of decreased body weight gains. No gross lesions were noted at necropsy. Bone marrow hypoplasia and uterus, ovary, and occasional mammary gland atrophy were seen primarily at the highest concentration, but also at 10,000 ppm *m*-Cresol/*p*-Cresol. Atrophy and regenerative changes in the nasal epithelia and forestomach were noted; this was presumably a direct result of the irritant effects of the chemical or its vapor.

The overall conclusion of this NTP study was that Cresol isomers produced a generally similar pattern of toxicities in rats and mice. Dietary concentrations of 3,000 ppm appeared to be minimal effect levels for increases in liver and kidney weights and deficits in liver function. Histopathologic changes, including bone marrow hypocellularity, irritation to the gastrointestinal tract and nasal epithelia, and atrophy of female reproductive organs, occasionally occurred at 10,000 ppm, but were more common at the high concentration of 30,000 ppm (NTP 1992a).

*o-Cresol.* Hornshaw et al. (1986) reported that 28-day feeding studies were conducted in mink by administering *o*-Cresol in the diet. Groups of 10 mink (5 male/5 female) received 0, 240, 432, 778, 1400, or 2520 mg/kg of *o*-Cresol in their feed. Doses were calculated to be 0, 35, 80, 125, 200, and 320 mg *o*-Cresol/kg body weight per day in males, and 0, 55, 120, 190, 300, and 480 mg *o*-Cresol/kg body weight per day in females. No deaths occurred and no clinical signs of toxicity were observed. Mink in the high-dose group had reduced feed consumption during the first week and body weight gains were decreased over the course of the study as compared to controls.

Hematologically there were decreases in red blood cell counts at doses  $\geq 1400$  mg *o*-Cresol/kg and in hemoglobin at the 2520 mg *o*-Cresol/kg. No lesions were found at gross necropsy. Liver to body weight and heart to body weight ratios were increased at  $\geq 432$  mg/kg and 2520 mg/kg, respectively. A NOEL of 240 mg *o*-Cresol/kg diet was identified for male and female mink in this study.

These authors also conducted 28-day feeding studies in ferrets by administering *o*-Cresol in the diet. Groups of five ferrets of each sex were fed diets containing 0, 432, 778, 1400, 2520, or 4536 mg/kg of *o*-Cresol. Doses were calculated to be 0, 45, 85, 140, 290, and 400 mg *o*-Cresol/kg body weight per day in males, and 0, 80, 150, 240, 530, and 720 mg *o*-Cresol/kg body weight per day in females. No deaths occurred and no clinical signs of toxicity were observed. Ferrets in the high-dose group had slightly reduced feed consumption, but no effect on body weight gains were noted. Hematologically, red blood cell counts were decreased at a dose of 4536 mg *o*-Cresol/kg. No lesions were found at gross necropsy. Liver to body weight and heart to body weight ratios were increased at  $\geq 1440$  mg/kg diet and 4536 mg/kg diet, respectively. A NOEL of 778 mg *o*-Cresol/kg diet was identified for male and female ferrets in this study (Hornshaw et al. 1986).

In a study reported by Chemical Industry Institute of Toxicology (CIIT) (1983), female B6C3F1 mice (8 to 10 weeks of age) received *o*-Cresol at calculated doses of 0, 6.5, 32.5, 65, or 130 mg/kg/day ad libitum in drinking water for 14 days. No immunotoxicity was seen in any parameter tested (changes in hematological values, lymphoid organ weights, altered lymphoid morphology, and cell or humoral-mediated immune function). Host resistance to challenge with tumor cells and *Listeria monocytogenes* was performed. No changes in immune function were reported and *o*-Cresol-exposed mice had the same mortality or tumor frequency following the challenge as did control animals. The threshold for immune responses was greater than 130 mg/kg/day.

*p*-Cresol. Altman et al. (1986) investigated the effects of the antioxidant, 3-tert-butyl-4-hydroxyanisole (BHA) and *p*-Cresol in the forestomach of rats. In a short-term (not further defined) feeding study, groups of 5 to 10 male and female rats were fed a powdered diet containing 2% BHA or 2% *p*-Cresol. In contrast to BHA, *p*-Cresol did not induce forestomach lesions.

Kawabe et al. (1994) reported on the combined effects of *p*-Cresol and NaNO<sub>2</sub> on cell proliferation in the upper digestive tract using male F344 rats. Groups of five rats were given either 2.0% *p*-Cresol or basal diet alone or in combination with 0.3% NaNO<sub>2</sub> for 4 weeks. The body weights of animals treated with *p*-Cresol or NaNO<sub>2</sub> individually were reduced 10% to 33% compared to those animals receiving basal diet alone. Rats treated with *p*-Cresol had a significant increase in the thickness of the forestomach mucosa in the prefundic or mid regions. In the esophagus, mucosal thickness or labeling indices were significantly increased in rats treated with *p*-Cresol or *p*-Cresol along with NaNO<sub>2</sub>.

*Thymol*. The Osaka Municipal Hygienic Laboratory (1953) reported a study in which two mice (sex and strain not specified) per group were fed a diet containing 10 or 30 mg Thymol and a third group of three mice was fed a diet containing 50 mg Thymol for 30 days; Thymol was finely ground and added to the feed. The animals received vegetables and water in addition to the basic diet. A control group of two animals was given feed that did not contain Thymol. Body weights and feed consumption were measured during the study.

Only the control animals and one animal from the 10 mg dose group survived until the study termination. The remaining 10 mg dose group animal died on day 27. The animals of the 30 mg dose group died on days 6 and 7. The animals of the 50 mg dose group died on days 5, 5, and 6. Body weights decreased in all dose groups as compared to controls (Osaka Municipal Hygienic Laboratory 1953).

*o*-Cymen-5-ol. The Osaka Municipal Hygienic Laboratory (1953) reported a study in which two mice (sex and strain not specified) per group were fed a diet containing 10, 30, or 50 mg *o*-Cymen-5-ol and a fourth group of three mice was fed a diet containing 80 mg *o*-Cymen-5-ol for 30 days; the *o*-Cymen-5-ol was finely ground and added to the feed. The animals received vegetables and water in addition to the basic diet. A control group of two animals received food that did not contain *o*-Cymen-5-ol. Body weights and feed consumption were measured during the study.

All animals of the control, 10 mg, 30 mg dose groups and one animal of the 50 mg dose group survived until the study termination. The other animal of the 50 mg dose group died on day 29. The animals of the 80 mg dose group died on days 10, 11, and 15. No variations in body weights or feed consumption were observed in mice fed 10 mg *o*-Cymen-5-ol. In the 30 and 50 mg dose groups, feed consumption was constant; body weights decreased temporarily in the 30 mg dose group and gradually throughout the study in the 50 mg dose group. The decrease in body weights for mice of the 50 mg dose was considered a toxic effect. No abnormalities in appearance or behavior were observed (Osaka Municipal Hygienic Laboratory 1953).

### Short-Term Dermal Toxicity

*PCMC*. New Zealand white rabbits, 50 per sex, received cutaneous applications of 0, 10, 40, and 160 mg/kg/day PCMC 5 days per week for 3 weeks on a shaved area of the back (Mobay Chemical Co. 1980). Irritation and erythema were observed, but there were no PCMC-related systemic effects as compared to controls.

*m*-Cresol. The effect of *m*-Cresol on mouse hair pigmentation was studied. *m*-Cresol was applied to the epilated or clipped area on the backs of five female CBA/J agouti mice. A mist of 0.5% *m*-Cresol in acetone was applied from a syringe for 6 weeks (three times weekly). Weekly observations were made for 6 months. Hair and skin samples were evaluated microscopically and no effects were observed (Shelley 1974).

*o*-Cresol. The effect of *o*-Cresol on mouse hair pigmentation was studied. *o*-Cresol was applied to the epilated or clipped area on the backs of five female CBA/J agouti mice. A mist of 0.5% *o*-Cresol in acetone was applied from a syringe for 6 weeks (three times weekly). Weekly observations were made for 6 months. Hair and skin samples were evaluated microscopically and no effects were observed (Shelley 1974).

*p*-Cresol. Shelley (1974) studied the effect of *p*-Cresol on mouse hair pigmentation, but effects on the skin were noted. *p*-Cresol was applied to the epilated or clipped area on the backs of 5 female CBA/J agouti mice and 30 male C57BL/6J black mice. A mist of 0.5% *p*-Cresol in acetone was applied from a syringe for 6 weeks (three times weekly). Weekly observations were made for 6 months. Hair and skin samples were evaluated microscopically.

At 0.5% *p*-Cresol, hair depigmentation was seen in black and agouti mice at 2 weeks, 4 weeks, and 6 months. In agouti mice, the tip of the hair was unaffected by *p*-Cresol and retained its darker pigmentation. In the black, male mice patches of hair pigment loss were induced by topical application of *p*-Cresol on the tip and hair shaft; depigmentation of the epidermis was also observed, especially on the tail. *p*-Cresol was toxic when large amounts (exact amount not stated) were painted on the skin. Repeated application had a local caustic and erosive effect, which was not observed in the agouti mice (Shelley 1974).

### Short-Term Inhalation Toxicity

*o*-Cresol. Cats exposed to 9 to 50 mg/m<sup>3</sup> of *o*-Cresol for 2 to 6 h/day for 1 month or more were reported to have inflammation and irritation of the upper respiratory tract, pulmonary edema, and hemorrhage and perivascular sclerosis in the lungs (U.S. Department of Health and Human Services 1992).

The WHO (1995) reported a study in which mice were exposed to a mixture of *o*-Cresol aerosol and vapor for 1 month (2 h/day for 6 days a week) with an average concentration of 50 mg/m<sup>3</sup>. No deaths were recorded. Clinical signs of toxicity were limited to respiratory irritation at the start of exposure followed by hypoactivity lasting until the end of the daily exposure. The tails of some animals "mummified" and fell off after 18 to 20 days. Body weight gains were slightly reduced compared to controls. Microscopically there were lesions in the respiratory tract (including edema, cellular proliferations, and small lung hemorrhages). Other lesions such as degeneration of heart muscle, liver, kidney, and nerve cells and glial elements of the central nervous system (CNS) were reported.

*Carvacrol*. Rats (three per group) were exposed to  $2.1 \times 10^{-8}$  M of Carvacrol for 4 or 8 weeks, after which the animals were killed. The number of hours and days of exposure were not given. The study examined the distribution of changes in the mitral cells of the olfactory bulbs following exposure to different odors, rather than on the nature and progression of the morphological alternations. The principal microscopic morphological changes noted were a darkening and shrinkage of the cell bod-

ies (both cytoplasm and nucleus). Moderate to dense selective degeneration was noted in the olfactory bulbs of rats (Pinching and Doving 1974).

### Short-Term Parenteral Toxicity

*PCMC*. Wein (1939) dosed three rabbits by subcutaneous injection with 5 ml/day of 0.25% PCMC in saline for 4 weeks. Survival was 100% and pathology was noted.

This author also dosed five albino rats by subcutaneous injection with 72 mg/kg PCMC as a 0.4% aqueous solution daily for 2 weeks and five rats were used as controls. All animals survived until study termination. The only observation was that of a mild inflammatory reaction with some leucocyte infiltration at the site of injection (Wein 1939).

*o*-Cymen-5-ol. The Osaka Municipal Hygienic Laboratory (1953) conducted a study in which groups of one to three mice (sex and strain not specified) were dosed by ip injection with 0.1, 0.2, 0.3, 0.6, 0.7, 1.4, 1.7, or 3.0 g/kg *o*-Cymen-5-ol as an oil-in-water emulsion once daily for 22 days. Body weights were measured during the study.

All mice dosed with  $\geq 0.3$  g/kg *o*-Cymen-5-ol had an anxiety reaction after dosing, which was followed by a gait disturbance and death while in an immobile moribund state. Changes in body weights were not observed for mice dosed with 0.1 or 0.2 g/kg *o*-Cymen-5-ol, whereas a gradual decrease in body weights was observed for mice dosed with 0.3 g/kg *o*-Cymen-5-ol. Induration at the injection site was observed at day 10 for mice in the 0.1 and 0.2 g/kg dose groups. For mice of the 0.3 g/kg dose group, severe induration was observed at the injection site and gait disturbance and immobility persisted as of day 16 (Osaka Municipal Hygienic Laboratory 1953).

### Subchronic Oral Toxicity

*PCMC*. SPF rats, 20 per sex per group, were fed 150, 500, or 1500 ppm PCMC in feed for 13 weeks. Controls were fed untreated feed daily for 13 weeks (BAYER AG 1980a). Body weights of the 500 and 1500 ppm dose groups were lower than those for control animals, independent of dose. No dose-related changes were observed at necropsy or upon microscopic examination; survival was 100%. The no-toxic-effect concentration was 150 ppm.

*m*-Cresol, *o*-Cresol, *p*-Cresol (*NTP Study*). In a 13-week toxicity study conducted by the NTP, *o*-Cresol or *m*-Cresol/*p*-Cresol (60:40) were added to the diet of rats and mice. For F344/N rats, *o*-Cresol and *m*-Cresol/*p*-Cresol concentrations were 0, 1880, 3750, 7500, 15,000 and 30,000 mg/kg in the diet. For B6C3F1 mice, *o*-Cresol concentrations were 0, 1250, 5000, 10,000, and 20,000 mg/kg and *m*-Cresol/*p*-Cresol concentrations were 0, 625, 1250, 2500, 5000, and 10,000 mg/kg in the diet (NTP 1992a).

*o*-Cresol arm of the NTP study—mice. All mice survived the 13-week *o*-Cresol study.

Mean final body weights were significantly decreased in high-dose males and at the three highest female dose groups

as compared to controls. Male mice fed 1250, 5000, 10,000, and 20,000 ppm *o*-Cresol and female mice fed 5000, 10,000, and 20,000 ppm *o*-Cresol gained less weight than control mice. During the first week of the study, feed consumption was decreased in high-dose males and females. Clinical signs of toxicity such as hunched posture and rough hair coat were observed in all high-dose male mice and hunched posture was noted in one male given 10,000 ppm *o*-Cresol.

At study termination, relative kidney weights were increased significantly in high-dose females as compared to controls. Relative liver weights were increased for all male dose groups and the three highest female dose groups. High-dose males had significantly increased relative testis and thymus weights. The relative thymus weights in females were also increased. There were no biologically significant changes in the hematology, clinical chemistry, and urinalysis data. Histopathologically there was minimal forestomach epithelial hyperplasia in 4 of 10 males and 3 of 10 females in the high-dose group and this lesion was sporadically noted in lower dose groups. The study stated that the effect may have been a result of direct chemical irritation or secondary to decreased feed consumption. An evaluation of vaginal cytology revealed a lengthened estrous cycle in high-dose mice. No changes in male reproductive endpoints were considered biologically significant (NTP 1992a).

*o*-Cresol arm of the NTP study—rats. All rats given *o*-Cresol survived the 13-week exposure except one female receiving 30,000 ppm was missing on day 8.

Decreases in mean final body weights and mean body weight gains were statistically significant in male rats given 30,000 ppm and female rats given 15,000 or 30,000 ppm *o*-Cresol. During the first week of the study, feed consumption was decreased in high-dose rats. No clinical signs of toxicity were observed in rats given *o*-Cresol. At study termination, relative liver weights were significantly increased in the rats given 7500, 10,000, or 30,000 ppm *o*-Cresol. Relative kidney weights were increased in rats at the two highest doses. Relative testis weight was increased in high-dose male rats and relative thymus weights were significantly increased for males in the two highest dose groups. Urinalysis and hematology were unremarkable.

Histopathologically, there was an increased incidence of bone marrow hypocellularity in high-dose males and in females in the two highest dose groups. The changes were minimal to mild in severity and the study considered the changes secondary to the decreased weight gains and not an indicator of direct chemical toxicity. A lengthening of the estrous cycle was observed for *o*-Cresol high-dosed rats, but no histopathologic changes were found in the ovary or uterus.

*m*-Cresol/*p*-Cresol arm of the NTP study—mice. All mice survived the 13-week exposure to *m*-Cresol/*p*-Cresol.

Mean final body weights were significantly decreased in high-dose mice exposed to *m*-Cresol/*p*-Cresol and mean body weight gains were decreased significantly in high-dose males.

Feed consumption was slightly decreased in high-dose mice. Rough hair coat was noted in 3 of 10 high-dose females. Relative liver weights were significantly increased for mice of both sexes at 10,000 ppm and males exposed to 5000 ppm *m*-Cresol/*p*-Cresol.

Hematology and clinical chemistry were mostly unremarkable. There was an increased serum 5'-nucleotidase concentration in high-dose females and an increased serum sorbitol dehydrogenase concentration in high-dose males that suggested hepatic damage had occurred. There were no corresponding microscopic liver lesions. Male mice at the two highest doses and female mice at the three highest doses had hyperplasia of the olfactory epithelium.

*m*-Cresol/*p*-Cresol arm of the NTP study—rats. All rats survived the 13-week exposure to *m*-Cresol/*p*-Cresol.

Mean final body weights were significantly decreased in rats exposed to *m*-Cresol/*p*-Cresol at the two highest concentrations. Mean body weight gains were decreased significantly in high-dose males and the two highest dose female groups. During the first week of the study, feed consumption was slightly decreased in high-dose rats. Rough hair coat was noted in all high-dose rats and thin appearance was noted in all high-dose female rats. Relative liver weights were significantly increased for rats of both sexes at the three highest doses. Relative kidney weights were significantly increased in high-dose females and males in the three highest dose groups. Relative testis weights were increased in males at the two highest dose groups.

Hematology results were mostly negative; serum alanine aminotransferase and sorbitol dehydrogenase (males) concentrations were increased in high-dose rats at day 5. An accumulation of bile acids in high-dose rats was considered evidence of decreased hepatocellular function resulting from Cresol ingestion. Urinalysis showed no clear evidence of renal injury. Dose-related hyperplasia of the olfactory epithelium was noted in all dose groups. Minimal hypocellularity of bone marrow was noted in high-dose females and males in the two highest dose groups. Minimal to mild uterine atrophy was observed in females in the two highest dose groups and a lengthened estrous cycle was noted in all dosed females.

The overall conclusion of this NTP study was that Cresol isomers produced a generally similar pattern of toxicities in rats and mice. Dietary concentrations of 3000 ppm appeared to be minimal effect levels for increases in liver and kidney weights and alterations in liver function. Histopathologic changes, including bone marrow hypocellularity, irritation to the gastrointestinal tract and nasal epithelia, and atrophy of female reproductive organs, occasionally occurred at 10,000 ppm, but were more common in the high-dose. Based on all the effects, a no observed adverse effect level (NOAEL) in rats of 3750 ppm diet was identified for *o*-Cresol. However, for *m*-Cresol/*p*-Cresol mixture the lowest dose tested resulted in changes in clinical chemistry and hyperplasia, therefore a threshold dose could not be established in rats. A NOAEL in mice of 1250 ppm and 625 ppm can be

identified for mice exposed to *o*-Cresol and *m*-Cresol/*p*-Cresol, respectively (NTP 1992a).

***m*-Cresol.** Dietz and Mulligan (1988b) evaluated the toxicity of *m*-Cresol using Sprague-Dawley rats in a 13-week study. *m*-Cresol was administered by gavage in corn oil at 0, 50, 150, or 450 mg/kg/day to rats (30/sex/group). The only clinical signs observed were lethargy, tremors, hunched posture, and rough hair coats in some male and female rats in the highest dose group. No clinical signs of toxicity were observed in rats treated with the mid or low dose of *m*-Cresol. Male rats in the mid-dose group had approximately a 10% to 15% weight gain reduction but this was not observed in females. In the high-dose group (450 mg *m*-Cresol/kg/day), male and female rats had a 20% to 25% reduction in body weight gains, respectively. One male rat in the highest dose group died. Clinical chemistry, hematology, and urinalyses parameters were not affected by *m*-Cresol treatment. There was no evidence of gross or histopathologic lesions in rats of any *m*-Cresol dose group.

***o*-Cresol.** Savolainen (1979) studied the toxicity of *o*-Cresol on the rat brain with special attention to glial cells. Forty male Wistar rats (average weight  $231 \pm 26$  g) were given drinking water with 2.8 mmol of *o*-Cresol per liter for 5, 10, 15, or 20 weeks and were then killed. Forty similar rats (average body weights of  $225 \pm 20$  g) were used as controls. Water consumption and weight gain were monitored weekly. Glial cells were isolated from five rats per group and the remaining rats were used to obtain cerebral samples that were homogenized in 0.1 M phosphate buffer at pH 7.4. Total cerebral RNA and glutathione concentrations were determined. Acid proteinase, NADPH-diaphorase, superoxide dismutase, and the azoreductase activities were determined.

*o*-Cresol treated rats gained weight slowly, but were not significantly different as compared to control rats (by week 20, control rats and *o*-Cresol treated rats weighed  $451 \pm 60$  and  $418 \pm 50$  g, respectively). Water consumption rates in *o*-Cresol treated rats was initially significantly increased (at week 4,  $48 \pm 4$  ml/rat/day in treated rats as compared to  $40 \pm 5$  ml/rat/day in controls), but by week 20, control rats drank more water than treated rats ( $49 \pm 4$  ml/rat/day in controls as compared to  $42 \pm 3$  ml/rat/day in treated rats). No significant differences in water consumption were observed at weeks 10 and 15. The cumulative dose at weeks 4, 10, 15, and 20 was 12.7, 26.8, 36.7, and 46.5 mmol/kg body weight, respectively.

At the end of the experiment, cerebral samples from five *o*-Cresol-treated rats had decreased glutathione concentration ( $1.4 \pm 0.2$  in treated rats versus  $1.6 \pm 0.1$  nmol/mg protein in controls) and azoreductase activity ( $26.8 \pm 7.9$  in treated rats versus  $38.2 \pm 9.9$  mmol/min  $\times$  mg protein in controls). There was an increase in cerebral RNA content in *o*-Cresol-treated rats observed only at week 4 ( $14.1 \pm 0.4$  in treated rats versus  $13.4 \pm 0.6$  in controls). There were no significant differences between *o*-Cresol-treated rats and controls in terms of acid proteinase, NADPH-diaphorase, and superoxide dismutase activity.

In *o*-Cresol-treated rats, glial cells had significant increases in acid proteinase and 2', 3'-cyclic nucleotide 3'-phosphohydrolase activities at the 20th week of exposure; however, there was no change in the glial cell glutathione concentration. The intestines of *o*-Cresol-treated rats appeared similar to controls, which could justify the assumption that the acute effects at the present dose are small. The authors concluded that "peroral intake of *o*-Cresol contaminated water is potentially toxic to rats if exposure continues for several weeks whereas the cresolic compounds may not accumulate in the body" (Savolainen 1979).

Dietz and Mulligan (1988a) evaluated the toxicity of *o*-Cresol in Sprague-Dawley rats in a 13-week study. *o*-Cresol was administered by gavage in corn oil at 0, 50, 175, or 600 mg/kg/day. Rats in the highest dose group had dyspnea, lethargy, and ataxia accompanied by tremors and/or convulsions. Rats in the mid-dose group occasionally were lethargic and had slight tremors, but adverse clinical signs were transient. Weight gain was decreased by 10% in male rats in the high-dose group after 13 weeks. In the high-dose group, 19 females and 9 males, out of 28 rats per sex, died. No treatment-related pathology was noted in any *o*-Cresol-treated rats. Clinical chemistry, hematology, and urinalysis were not affected by *o*-Cresol treatment. The study concluded that 175 mg/kg/day for *o*-Cresol administered to Sprague-Dawley rats for 13 weeks appeared to be the threshold at which no significant toxicological effects were observed, with the exception of transient weight and weight gain effects in males and infrequent signs of tremors or coma, which subsided after 1 h.

***p*-Cresol.** Hirose et al. (1986) examined the induction of proliferative lesions of the forestomach in hamsters. Hamsters were used because they are more sensitive than rats to inducers of forestomach lesions. Male, Syrian golden hamsters were dosed with 1.5% *p*-Cresol, which was added to their basal diet for 20 weeks. The dose was approximately 25% of the LD<sub>50</sub> for rats (LD<sub>50</sub> not stated). There were 15 hamsters per group, approximately 7 weeks old, and weighing  $212 \pm 25$  g. Control hamsters were fed a basal diet only. Diet and water were available ad libitum. A labeling (thymidine) index was used in pyloric regions of the glandular stomach.

The test group had a greater incidence of mild and moderate forestomach hyperplasia as compared to the control. Treated hamsters had increased liver weight gain ( $4.4 \pm 0.6$  compared to the control gain of  $3.8 \pm 0.4$  g/100 g of body weight), mild forestomach hyperplasia (occurring in test versus control hamster groups, 100% versus 46.7%, respectively), and of moderate forestomach hyperplasia (occurring in test versus control hamster groups, 66.7% versus 6.7%, respectively). Severe forestomach hyperplasia and papillomatous lesions were not observed in *p*-Cresol or control groups. The labeling index in pyloric regions of the glandular stomach was slightly increased as a result of *p*-Cresol treatment. Inflammation, hyperplasia, or tumorous lesions were not observed in the urinary bladder (Hirose et al. 1986).

Dietz and Mulligan (1988c) evaluated the toxicity of *p*-Cresol administered by gavage for 13 weeks to Sprague-Dawley rats. Thirty rats per sex were used for each dose group tested. A

separate group of 10 male and 10 female rats were used for baseline hematology and clinical chemistry. *p*-Cresol was diluted in corn oil for a dosing volume of 5 ml/kg for each dose group of 0, 50, 175, and 600 mg/kg/day. An interim kill was performed on some rats at week 7.

Three high-dose (600 mg/kg/day) females died in the first 3 days of the study. Male and female rats in the high-dose group exhibited lethargy, excessive salivation, tremors and occasional convulsions, central nervous system depression, and comas throughout the study. These clinical signs were not observed at lower doses and no other treatment-related signs of toxicity were observed. Three female rats in the high-dose group died within the first 3 days of the experiment. Significant dose-related decreases in body weight gains occurred in high-dose males during weeks 1 to 13 and in mid-dose males during weeks 1 to 3. High-dose and mid-dose male body weight gains were decreased approximately 25% and 7%, respectively. High-dose females had significantly decreased body weight gains (15%) during weeks 1 to 7 and 10 and 13.

Serum transaminase activity was significantly increased for high-dose females and hypercholesterolemia was observed in this group and possibly in high-dose and mid-dose males. Total protein and relative liver weights were significantly increased in high-dose males. Histologic lesions of the liver were not observed. No ocular lesions were attributable to *p*-Cresol exposure by gavage administration. Renal injury was suggested by organ weights and light microscopic changes. Slight increases in the incidence of chronic nephropathy were noted in all male dose groups but were significantly increased at the low and high doses. Significantly increased kidney weights were observed in mid-dose and high-dose male rats and high-dose females. Clinical pathology did not clarify these observations.

A dose-related anemia occurred in female rats at the mid and high doses. This effect was slight, and compensatory physiologic mechanisms such as increased nucleated red blood cells (RBCs), retulocytosis, and macrocytosis were not evident. Based on the results of the study, *p*-Cresol was hepatotoxic, nephrotoxic, and induced a mild anemia; 50 mg/kg/day appeared to be the threshold at which no toxic effects were observed (Dietz and Mulligan 1988c).

**Thymol.** A 19-week subchronic toxicity study of Thymol in weanling Osborne-Mendel rats was done. There were 10 rats (5 male/5 female) per dose group. Rats were given Thymol at 0, 1000, and 10,000 ppm in their diet. Body weights, food intake, and general condition were recorded weekly. Hematology examinations were made at the termination of the study. The liver, kidneys, spleen, heart, and testes were weighed. The tissues of all rats were examined grossly at death. There was no effect noted in either dose group as compared to controls (Hagan et al. 1967).

***o*-Cymen-5-ol.** Four or five white rats (sex and strain not specified) per group were fed a diet for 90 days to which an emulsified mixture containing 10 g *o*-Cymen-5-ol was added (Osaka Municipal Hygienic Laboratory 1954). The amount of

emulsified mixture added to the basic diet was adjusted so that the groups were dosed with 1, 10, 100, or 200 mg *o*-Cymen-5-ol per 100 g body weight. A control group of five rats was fed diet alone. There was a nontreatment period of 10 days after the termination of dosing in which the test animals were fed control diet. General observations were made, body weights were measured, and urinalysis (using 24-h urine samples) were performed every 15 days. For body weight determinations, the body weights on day 1 for each group was defined as 100, and the weight index (percentage of body weight before and after feeding to that on day 1) was calculated for all rats. The group mean weight index was used for comparison at each observation period. At the study termination, the animals were necropsied and gross and microscopic examination of tissues was performed.

No animals died on study. Motor activity was decreased and somnolence was observed for animals of the 200 mg group during the first observation period. Although the haircoat was not affected, the observations were attributed to *o*-Cymen-5-ol administration. These signs were only seen during the first period, suggesting that the rats acquired a tolerance to *o*-Cymen-5-ol. No general observations as to behavior or appearance were made for any other dose group.

During the first and third observation periods, body weights increased for animals in all the dose groups except the 200 mg dose group; the decrease in body weights for the 200 mg dose group were considered to be due to test article administration. During the second observation period, body weights increased in the 200 mg dose group, but there were apparent differences among the higher dose groups (100 and 200 mg), the lower dose groups (1 and 10 mg), and the controls. Body weight gains were normal during the fourth observation period and throughout the remainder of the study. During the nontreatment period, the body weights for the rats of the 100 and 200 mg groups rapidly increased.

Urine was alkaline or weakly alkaline for all groups throughout the study. Protein was detected in a dose-related manner in the urine. Occasionally occult blood was positive, even in the low-dose groups, but these changes were transient. Other results obtained during urinalysis were not considered to be dose-related.

No gross observations were made at necropsy. Histologically, there were no treatment-related differences between treated and control groups (Osaka Municipal Hygienic Laboratory 1954).

### Subchronic Inhalation Toxicity

Kurlyandskiy et al. (1975) exposed rats to Mixed Cresols by inhalation for 90 days. The experiment was done using three groups with six rats per dose group; rats were exposed to 0, 0.005, or 0.05 mg/m<sup>3</sup> Mixed Cresols. The particle size was not stated. Treatment-related effects included CNS excitation, denaturation of lung protein, and decreased body weight gains in the high-dose group. The maximum permissible concentration of Mixed Cresols was 0.005 mg/m<sup>3</sup>.

RIFM (2001c) reported a study in which rats were exposed to *o*-Cresol for 6 h/day for 2 months and at 4 or 5 times/week for 2 months. Survival, body weights, clinical signs, nervous activity, blood analysis, and liver function were measured; necropsy and microscopy were performed. At a concentration of 9 mg/m<sup>3</sup> blood effects, clinical signs, neurological effects, and changes in leukocytes, spinal cord smears, nervous activity, and liver function were observed. In a similar experiment using guinea pigs, the same concentration of exposure to *o*-Cresol was used. Changes were seen in hemoglobin concentrations and electrocardiograms (EKGs) from guinea pigs exposed to 9 mg/m<sup>3</sup>.

### Chronic Oral Toxicity

The NTP concluded that there was little evidence to suggest a significant increase in toxicity with longer exposures of *m*-Cresol, *o*-Cresol, and *p*-Cresol than 13-week studies as compared to the effects seen with similar doses in the 28-day studies (NTP 1992a). However, NTP has begun an oral Toxicology/Carcinogenesis study on Mixed Cresols (NTP 2002).

At this time, therefore, studies on the chronic oral toxicity of the ingredients in the Cresol family were available only on PCMC.

### PCMC

Bor:WISW(SPF Cpb) Wistar rats, 60 animals/sex/group, were fed 400, 2000, or 10,000 ppm PCMC for 2 years, with the exception of 10 animals/sex/group that were necropsied after 53 weeks (Bayer AG 1992). A control group of 60 animals/sex was fed a diet that did not contain PCMC for 2 years, again with the exception of 10 animals/sex that were necropsied after 53 weeks (dosages were determined based on results of previous short-term and subchronic studies summarized previously in this report).

The animals were observed at least twice daily, with detailed weekly evaluations; body weights were also measured weekly. Feed consumption was determined weekly for 13 weeks, after which it was determined at 4-week intervals. Water intake was determined at 4-week intervals over the entire study. Ophthalmological evaluations were performed on 20 animals per sex per group prior to study initiation and then on 20 animals per sex of the control and 10,000 ppm groups after 52 and 104 weeks of dosing. Blood samples were obtained for examination from 10 animals per sex per group after 27, 52, 79, and 104 weeks of dosing. Urinalyses were performed during weeks 26/27, 51/52, 78/79, and 103/104.

Averaged over 2 years, the males and females of each dose group ate the following quantities (per day) of PCMC: 400 ppm group, 21 and 27.7 mg, respectively; 2000 ppm group, 103.1 and 134.3 mg, respectively; and 10,000 ppm group, 558.9 and 743.5 mg, respectively. Body weights of female rats of all dose groups and of male rats of the 10,000 ppm dose group were significantly decreased compared to controls throughout the study. Body weights of the males of the 400 and 2000 ppm dose groups

were comparable to control values. Mean feed intake per animal and day was comparable between males of all dose groups and controls but was slightly less for females of all dose groups compared to the controls. When related to body weights, the mean feed intake of both males and females of the 10,000 ppm dose groups was increased compared to controls. Male rats of the 10,000 ppm group had increased mean water intake per animal per day, and the water intake per kg body weights for females of the 10,000 ppm group was also increased compared to controls.

Abdominal circumference was significantly reduced for female animals of the 2000 and 10,000 ppm dose groups, and the frequency of a poor general condition was statistically increased for females of the 10,000 ppm dose group. Ophthalmological examination did not indicate any ocular toxicity. There was no significant difference in mortality between animals of the test and control groups.

PCMC-related clinical chemistry or hematological changes were limited to reduced serum potassium and phosphate concentrations (compared to control values) for both males and females of the 10,000 ppm group. Urinalyses results included a decrease in total protein excretion for males and females fed 10,000 ppm PCMC; males of this group also had a reduced urinary density, often in conjunction with a slightly enhanced urinary volume.

No significant findings were reported in the rats at the interim necropsy after 53 weeks; absolute and relative organ weights did not differ significantly between test and control groups. At study termination, necropsy findings included deformation of the kidneys in 6 of the 44 surviving males of the 10,000 ppm group. Relative kidney weights of males of the 2000 ppm group and males and females of the 10,000 ppm group were slightly increased compared to control values.

No microscopic lesions were observed at interim necropsy. Females of the 2000 ppm dose group that died during the study had dilated ducts of the mammary gland. At study termination, an increased incidence of renal papillary necrosis, cortical tubular dilations, and cortical fibrosis was observed in male rats of the 10,000 ppm group. Females of the 2000 and 10,000 ppm groups that died on study had a greater incidence of pituitary adenoma, a finding that was not observed at interim necropsy (only preliminary microscopic information was available from rats of the final necropsy). The authors stated that the NOEL was 2000 ppm PCMC (Bayer AG 1992).

### Ocular Irritation

The Mellon Institute of Industrial Research (1949) reported the effects of instillation of a dilution of *m*-Cresol in propylene glycol in the rabbit eye. At 5% dilution, *m*-Cresol in propylene glycol caused severe damage to the cornea of the rabbit eye, but a 1.0% dilution had no effect. *m*-Cresol was rated a 9 out of 10 in the eye burn ratings system.

Fiebig et al. (1972) instilled PCMC, 0.05% or 0.1% in 0.9% saline, into the conjunctival sac of the eyes of rabbits (number of animals not stated) by applying 20 drops per minute for 10 min;

the eyes were rinsed with saline after 15 min. Fluorescein staining was used. In the animal(s) dosed with 0.05% PCMC, the "cornea" (sic) was reddened for 15 min to 4 h after application and the eye was normal after 24 h. In the animal(s) dosed with 0.1% PCMC, the eye was inflamed after 48 h and clear after 106 h.

Davies (1973) applied soft contact lenses that were stored in bactericidal strength solution (isotonic solution, pH 7.0, containing 0.1% PCMC) to the eyes of 13 rabbits for 6 h per day. The eyes were not rinsed. PCMC produced severe irritation after a few days.

FDRL (1975d) reported that acute eye irritation was produced in 9 albino rabbits using 0.1 ml of undiluted *o*-Cresol. Group I had six rabbits and their eyes were unrinsed following the instillation of the test material. Group II had three rabbits and eyes were rinsed 4 s following the instillation of the test material. Observations were made up to 72 h post application. The eye was scored for damage to the cornea, iris, and conjunctivae. Corneal, iridial, and conjunctival effects were observed in all animals throughout the 72-h observation period. It was concluded that *o*-Cresol is a severe eye irritant to the rabbit eye with or without a washout 4 s after installation.

The Dow Chemical Company (1978) reported that direct contact of Mixed Cresols to the eyes of an albino rabbit has an extremely severe effect. Tissue destruction was reported, leading to permanent vision impairment. The authors expressed the view that superficial rinsing of a contaminated eye would not be effective in reducing the degree of injury.

The EI DuPont de Nemours & Co. (1983) evaluated acute eye irritation of 0.1 ml of undiluted *m*-, *o*-, or *p*-Cresol using six rabbits. Observations were made for 72 h post application. Damage to the cornea, iris, and conjunctivae was scored. The *m*-Cresol irritation score was 87.3/110 at 24, 48, and 72 h with most of the damage to the cornea. The *o*-Cresol irritation score was 91.3/110 after 24 h with most of the damage to the cornea. The irritation was reduced to 82.7/110 at 72 h. The *p*-Cresol irritation score was 84.7/110 after 24 h with most of the damage to the cornea. The irritation was increased at 72 h and was 93.0/110.

MB Research Labs (1996) tested the ocular irritation caused by an OTC topical cream containing 0.032% Chlorothymol using the chorioallantoic membrane (CAM) vascular assay. Fertile, white Leghorn eggs (40) were incubated for 14 days. On day 4, the eggs were removed and a small hole was drilled into the narrow end of each and approximately 2.5 ml of albumen was removed and the hole was sealed using collodion adhesive. A rectangular window was cut in the shell directly above the developing embryo and the opening was covered with transparent tape and returned to the incubator.

On day 14, the eggs were removed and a Teflon ring was placed on the CAM and 40  $\mu$ l of the test material was pipetted into the ring. The window was resealed and eggs returned to the incubator for 30  $\pm$  5 min. The eggs were then removed and the CAM was exposed by removing the tape and portions of the shell. The condition of the CAM within the ring was evaluated,

and if there were any abnormalities outside the ring, the egg was not included in the calculations. Any vascular hemorrhage, capillary injection, and/or presence of ghost vessels was considered indicative of a positive response.

At concentrations of 75%, 80%, 90%, and 100% there were 1 of 10, 1 of 10, 4 of 10, and 5 of 10 positive responses in eggs, respectively. The  $RC_{50}$  was calculated as 97%. An  $RC_{50}$  value greater than 3.0% indicates the chemical is a nonirritant. An  $RC_{50}$  value of less than 1.0% classifies a chemical as an irritant. Therefore, the OTC topical cream containing 0.032% Chlorothymol was not considered an ocular irritant (MB Research Labs 1996).

In a study reported by CTFA (1980b), the ocular irritating ability of 0.1% and 1.0% *o*-Cymen-5-ol in Vaseline was determined in two groups of albino rabbits (nine animals/group). The sample of *o*-Cymen-5-ol employed in the study had purity of >99%. One-tenth gram of the test material was instilled once into one eye of each rabbit; the other eye served as untreated control. The Vaseline vehicle was also instilled into one eye of each of three animals. The eyes of the vehicle control group received no water rinse. Eyes of the treated animals received either no water rinse (three rabbits/group) or a water rinse 2 (three rabbits/group) or 4 (three rabbits/group) s after administration of *o*-Cymen-5-ol. Eye irritation was graded according to the evaluation system of Draize (Draize 1959) at 1 h, 4 h, and days 1 to 7 after instillation of the test substance. In the 0.1% treatment group, discharge and "barely perceptible" to "very slight" redness were observed in the conjunctivae under both rinse and no rinse conditions 1 and 24 h after treatment. This eye irritation had completely cleared by the 48-h reading.

In the 1.0% treatment group, the authors stated that very slight redness and discharge occurred in the conjunctivae of the unwashed eye after 1 and 4 h; no irritation was detected by the 24-h reading. No ocular irritation was noted in the rabbits receiving the water rinse. In the vehicle-control group, the authors stated that very slight redness and discharge were observed in the conjunctivae at the 1- and 4-h reading; this irritation dissipated within 24 h following treatment. It was the investigator's opinion that the ocular irritation produced by *o*-Cymen-5-ol was very low.

### Dermal Irritation

No animal dermal irritation studies were found on Sodium *p*-Chloro-*m*-Cresol, Chlorothymol, or Isopropyl Cresols. Dermal irritation of the other Cresols was evaluated using rabbit, guinea pig, or rat skin, in vivo or in vitro. These studies are presented in Table 7. These data demonstrate that the severity of skin reactions increased with concentration.

### Dermal Sensitization

No published animal dermal sensitization studies were found on Sodium *p*-Chloro-*m*-Cresol, Chlorothymol, Isopropyl Cresols, Mixed Cresols, or *m*-Cresol.

## COSMETIC INGREDIENT REVIEW

**TABLE 7**  
Dermal irritation studies

Chemical	Species/number	Methods/dose	Results	Reference
PCMC	Rabbits/2 per dose group	The trypan blue method was used to determine the dermal irritation potential of PCMC. Single application of 0.2% PCMC in normal saline or 0.4% or 0.8% PCMC in 1% Tween in normal saline. The site of application was four areas on the abdominal region. 0.4 ml injected intradermally within 10–15 min. Twenty min after dosing, 1 ml/kg of 1% trypan blue was injected intravenously and the color at the injection sites was observed for 3 h.	The maximal irritation score (scale not stated) was 4 for 0.2% and 0.4% and 8 for 0.8% PCMC.	Baichwal and Phadnis 1968
Mixed Cresols	Six albino rabbits	Mixed Cresols was applied and secured to abraded and intact skin. Dose was not stated.	After 24 h, the skin was severely red and swollen. The primary irritation score was 6.58, which classified Mixed Cresols as a primary irritant. At 72 h, moderate to severe redness and swelling were observed.	Dow Chemical Company 1978
Mixed Cresols	Unstated animal skin	Mixed Cresols was tested for corrosivity in animal skin (species not stated).	Two of two tests were positive with necrosis observed. Mixed Cresols was considered a corrosive material and must be labeled “corrosive” with a DOT label for shipping within the United States.	Koppers Company, Inc. 1975
<i>m</i> -Cresol, <i>o</i> -Cresol, and <i>p</i> -Cresol	Mice (5 female and/or 30 male)	<i>m</i> -Cresol, <i>o</i> -Cresol, and <i>p</i> -Cresol (0.5% of each isomer) were applied by syringe to epilated or clipped areas on the backs of 5 female CBA/J agouti mice and/or 30 male C57BL/6J mice 3 times weekly, for 6 weeks; vehicle was acetone. The skin and hair samples were examined microscopically.	After 6 months of application, there was no effect on the skin and hair using 0.5% <i>m</i> -Cresol and 0.5% <i>o</i> -Cresol. However, repeated application of 0.5% <i>p</i> -Cresol had depigmentation and a local caustic, erosive effect on black mice.	Shelley 1974
<i>m</i> -Cresol	Five rabbits	10% <i>m</i> -Cresol (0.01 ml) applied to bellies of 5 rabbits.	10% <i>m</i> -Cresol (in acetone) caused severe erythema in 2 animals and erythema and moderate edema in 3 of 5 animals tested. <i>m</i> -Cresol was graded as a 6 out of 10 in terms of irritation and produced necrosis of the skin on the rabbit belly.	Mellon Institute of Industrial Research 1949

(Continued on next page)

**TABLE 7**  
Dermal irritation studies (*Continued*)

Chemical	Species/number	Methods/dose	Results	Reference
<i>m</i> -Cresol	Rabbits	<i>m</i> -Cresol dermal exposure over a 4-h period at a dose of 2830 mg/kg.	<i>m</i> -Cresol caused significant dermal corrosion within a 4-h period at a dose of 2830 mg/kg. The same dose was the LD <sub>50</sub> in rabbits when applied to the skin.	Vernot et al. 1977
<i>m</i> -Cresol	Six albino rabbits	Undiluted <i>m</i> -Cresol was used. Edema and erythema were scored on intact and abraded skin at 24 and 72 h. Primary skin irritation was scored on an 8.0 scale.	Edema and erythema were observed in all rabbits. The irritation score of <i>m</i> -Cresol was 8.0/8.0.	EI DuPont de Nemours & Co. 1983
<i>m</i> -Cresol/ <i>p</i> -Cresol	Albino rabbits	Skin irritation was evaluated after application of 0.5 ml (undiluted) <i>m</i> -Cresol/ <i>p</i> -Cresol solution. The solution was applied to the clipped and intact skin of male and female albino rabbits under a 1 × 1 patch for 4 h. Observations were noted at 4 and 24 h and up to 17 days.	At 4 h, necrosis and severe edema were noted, at 24 h eschar formation was observed, and the scab sloughed off in 14 to 17 days showing the injury in depth. The <i>m</i> -Cresol/ <i>p</i> -Cresol solution was classified as corrosive under these conditions.	Ferro Corporation 1974
<i>o</i> -Cresol	Six albino rabbits	Primary skin irritation caused by an undiluted solution of <i>o</i> -Cresol was evaluated using six albino rabbits. The back was shaved free of hair and 0.5 g or 0.5 ml of <i>o</i> -Cresol was introduced under a 1 × 1-inch gauze patch for 24 h after which the patch was removed.	Edema and erythema were observed in all rabbits. The irritation score of <i>o</i> -Cresol was 5.6. On the basis of this test, <i>o</i> -Cresol was considered a severe irritant to the rabbit skin.	FDRL 1975c
<i>o</i> -Cresol	Albino rabbits	<i>o</i> -Cresol (0.5 ml undiluted) was applied to the clipped and intact skin of male and female albino rabbits under 1 × 1-inch patch for 4 h and observations were recorded for 14 days.	At 4 h, necrosis and severe edema were noted, at 24 h, eschar formation was observed, and at 14 days there was slight loosening about the edges of the scab which showed the injury in depth. <i>o</i> -Cresol was classified as corrosive under these conditions.	Ferro Corporation 1974
<i>o</i> -Cresol	New Zealand albino rabbits	<i>o</i> -Cresol (0.5 g undiluted) was applied to the backs of clipped rabbits for 4 h under two 1 × 1-inch gauze patches. After 4 h the patches were removed and any remaining material was rinsed off.	<i>o</i> -Cresol produced destruction of the epidermis, dermis and subdermal fat layer in all of the test animals.	Scientific Associates Inc. 1976

(*Continued on next page*)

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**TABLE 7**  
Dermal Irritation Studies (*Continued*)

Chemical	Species/number	Methods/dose	Results	Reference
<i>o</i> -Cresol	Six albino rabbits	Primary skin irritation was evaluated. The backs were clipped free of hair and 0.5 g of 60% <i>o</i> -Cresol was introduced under a 1 × 1-inch gauze patch for 4 h after which the patch was removed and the backs were rinsed. The site was examined at 4, 24, and 48 h post-exposure.	Moderate to severe edema and erythema were observed in all rabbits at 4, 24, and 48 h post-exposure to 60% <i>o</i> -Cresol. Eschar formation was seen in 3 rabbits at 48 h. <i>o</i> -Cresol was considered corrosive to the skin of albino rabbits.	Biosearch, Inc. 1980
<i>o</i> -Cresol	Rabbits (unstated number)	Rabbits were dosed with 0.625 to 5 g/kg of <i>o</i> -Cresol. The study was primarily done to assess the dermal acute toxicity, but irritant effects were noted.	Moderate to severe skin irritation was noted. Ulcerated and thickened treated skin was observable at necropsy.	RIFM 1980a as cited in RIFM 2001c
<i>o</i> -Cresol	Rabbits (unstated number)	The dermal corrosive properties of <i>o</i> -Cresol were demonstrated using rabbits treated with 0.5 g or 0.5 ml <i>o</i> -Cresol under a 1 × 1 inch patch	<i>o</i> -Cresol caused significant dermal corrosion within a 4-h period.	Vernot et al. 1977
<i>o</i> -Cresol	Six albino rabbits	Primary skin irritation caused by undiluted <i>o</i> -Cresol (volume not stated) was evaluated using albino rabbits. Edema and erythema were scored on intact and abraded skin in 6 rabbits at 24 and 72 h.	Edema and erythema were observed in all rabbits. The irritation score of <i>o</i> -Cresol was 8.0/8.0.	EI DuPont de Nemours & Co. 1983
<i>o</i> -Cresol	Two guinea pigs	Two guinea pigs were observed during an <i>o</i> -Cresol LD <sub>50</sub> study and irritation was evaluated at a dose of 5 g/kg.	Guinea pigs had no irritation from a 5 g/kg dose of <i>o</i> -Cresol to the skin, but ulcerated and thickened treated skin at necropsy was noted.	RIFM 1980a as cited in RIFM 2001c
<i>p</i> -Cresol	Rabbits	The dose was the LD <sub>50</sub> in rabbits when applied to the skin. No other details given.	<i>p</i> -Cresol caused significant dermal corrosion within a 4-h period at a dose of 300 mg/kg.	Vernot et al. 1977
<i>p</i> -Cresol	Albino rabbits	Primary skin irritation caused by undiluted <i>p</i> -Cresol (volume not stated) was evaluated in albino rabbits. Edema and erythema were scored on intact and abraded skin in 6 rabbits at 24 and 72 h.	Edema and erythema were observed in all rabbits. The irritation score of <i>p</i> -Cresol was 8.0/8.0.	EI DuPont de Nemours & Co. 1983
Thymol and Carvacrol	Six to eight male and female Himalayan guinea pigs	0.025 ml of 0.03%, 0.1%, 0.3%, 1%, 3%, 10%, or 30% Thymol or Carvacrol was used. Prior to the induction procedure, Thymol or Carvacrol	After a single application the MIC was 3% Thymol or 3% Carvacrol, which was defined as producing mild erythema in at least 25% of the animals.	Klecak et al. 1977

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**TABLE 7**  
Dermal irritation studies (*Continued*)

Chemical	Species/number	Methods/dose	Results	Reference
		was applied with a pipette to a 2-cm <sup>2</sup> area on the clipped flank of guinea pigs, left uncovered, and reactions were scored after 24 h. Thymol and Carvacrol were evaluated for irritation during the induction phase using a 0.1-ml aliquot applied to an 8-cm <sup>2</sup> area on the clipped flank of guinea pigs per test group. The site was left uncovered and reactions were read after 24 h. A total of 21 daily applications were made.	The minimal irritating concentration was 3% Thymol or 0.3% Carvacrol, which produced erythema in at least 25% of the animals.	
<i>o</i> -Cymen-5-ol	Eight albino rabbits	Primary skin irritation using 0.1% and 1.0% <i>o</i> -Cymen-5-ol in rabbits. Patches containing the test materials and the Vaseline vehicle were applied to the clipped skin of each animal. Test sites on the eight animals were either abraded (four rabbits) or intact (four rabbits). After 24 h, the patches were removed and the skin reactions evaluated on a scale of 0.0 (no erythema or edema) to 8.0 (severe erythema and edema).	No irritation was observed to 0.1% or 1.0% <i>o</i> -Cymen-5-ol on either intact or abraded skin. Barely perceptible erythema was noted at the 72-h reading on the intact skin of one rabbit as a result of exposure to the Vaseline vehicle.	CTFA 1980c
<i>o</i> -Cymen-5-ol	Eight male albino rabbits	5% <i>o</i> -Cymen-5-ol in PEG 400 was used in the study. The back of each animal was clipped of all hair, and patches containing 0.3 ml of the test material were then applied to either intact (four rabbits) or abraded (four rabbits) skin. After 24 h, the patches were removed and the skin reactions evaluated on a scale of 0 (no erythema or edema) to 8.0 (severe erythema and edema). Skin responses were graded again after 72 h.	The Primary Irritation Index (PII), a value depicting the average score of each exposure group as a whole, was 0.0/8.0 for the vehicle control animals. The PII for the 5.0% treatment group was 0.06/8.0 indicating a "very low" degree of primary skin irritation.	CTFA 1976a
<i>o</i> -Cymen-5-ol	Three male albino guinea pigs	10% <i>o</i> -Cymen-5-ol (0.3 ml) in ethanol was applied every day for three days to the clipped flanks of each animal. Following each of the three 24-h exposures, skin reactions were graded on a scale of 0	The PII for ethanol and 10% <i>o</i> -Cymen-5-ol in ethanol were 0.0/4.0 and 0.22/4.0, respectively. The latter score was considered by the investigator to be indicative of a "very low" degree of primary skin irritation.	Magnusson and Kligman 1970

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**TABLE 7**  
Dermal irritation studies (*Continued*)

Chemical	Species/number	Methods/dose	Results	Reference
		(no reaction) to 4.0 (severe erythema and edema). It was not reported whether test sites were covered with an occlusive patch, whether skin was intact and/or abraded, or whether the same sites were used for each 24-h application.		
<i>o</i> -Cymen-5-ol	Five female Hartley guinea pigs	Occlusive wet patches with 0.1 ml of <i>o</i> -Cymen-5-ol (0.5%, 1.0%, 2.0%, and 4.0% <i>o</i> -Cymen-5-ol in 50% ethanol solution) were applied to the clipped flank of guinea pigs for 24 h. Reading were taken 24 h after patch removal.	No reactions were observed at any test concentrations for any animal.	Osaka Kasei Co., Ltd. 1992a
Carvacrol	In vitro	Carvacrol was evaluated for skin corrosivity in vitro. The parameters used to classify Carvacrol were the octanol-water partition coefficient, molecular volume, melting point, and pK <sub>a</sub> value.	Carvacrol had a primary irritation index of >4 and was subsequently classified as corrosive. Carvacrol was a borderline corrosive/non-corrosive chemical and structure-activity-relationship analysis was used to clarify its classification as a corrosive.	Barratt et al. 1998
Carvacrol	Ten rabbits	The irritancy effects of Carvacrol (5 g/kg) was evaluated on day 1, during a dermal LD <sub>50</sub> study.	Carvacrol at doses of 5 g/kg had irritant effects including severe redness (in 9/10 rabbits), moderate edema (in 3/10 rabbits) and severe edema (in 6/10 rabbits). Severe eschar formation and brown epidermis in the application area were noted in half the rabbits.	RIFM 1977 as cited in RIFM 2001a
Carvacrol	In vitro (rat skin)	Rat skin was used for an in vitro test to evaluate the corrosivity of Carvacrol (concentration not stated). Carvacrol was applied to rat skin disks for 2 h and 24 h.	Carvacrol was classified as "noncorrosive."	Fentem et al. 1998
Carvacrol	Ten rabbits	The irritancy of Carvacrol (2.5 g/kg) was evaluated on day 1 during a dermal LD <sub>50</sub> study.	Carvacrol at doses of 2.5 g/kg had irritant effects including severe redness and moderate edema (in 2/2 rabbits) and severe redness, moderate edema, and eschar at the exposure site (in 8/8 rabbits).	RIFM 1977 as cited in RIFM 2001a

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**TABLE 7**  
Dermal irritation studies (*Continued*)

Chemical	Species/number	Methods/dose	Results	Reference
Carvacrol	4 female New Zealand white rabbits	A 4-h semiocclusive patch test was conducted using rabbits. A 0.5-ml aliquot of Carvacrol was applied to the clipped dorsal skin. The skin was cleansed at patch removal and reactions were assessed at 1, 24, 48, and 72 h as well as 7 days after patch removal.	There were irritant effects in all rabbits tested. The average score for erythema was 4.0 and for edema there was insufficient data to calculate a score due to hardening of the treated site. Using the EEC classification system, Carvacrol was considered an irritant.	RIFM 1988 as cited in RIFM 2001a
<i>m</i> -Cresol, <i>o</i> -Cresol, <i>p</i> -Cresol, Thymol, and Carvacrol	Rabbit skin	Quantitative structure-activity relationships (QSARs) were used to predict the skin corrosivity. Data sets were analyzed by principal components analysis and by neural network analysis. Chemicals with predicted outputs below 0.5 were classified as noncorrosive and those with predicted outputs of 0.5 or greater were classified as corrosive (defined as causing irreversible destruction of skin tissue when applied to rabbit skin for a period up to 4 h).	The input variables gave predicted outputs on a scale of 0 to 1 with 1 being the most corrosive and 0 being noncorrosive. The chemicals <i>m</i> -Cresol, <i>o</i> -Cresol, <i>p</i> -Cresol, Thymol, and Carvacrol were all classified as corrosive with predicted outputs of 0.97, 0.98, 0.98, 0.79, and 0.94, respectively.	Barratt 1996

Dermal sensitization of the other cresols was evaluated in the guinea pig maximization test (GPMT), open epicutaneous test (OET), Freund's complete adjuvant (FCA) test, and Draize test.

Sensitization studies are summarized in Table 8. Most positive reactions were observed with higher concentrations of Cresol ingredients.

### Phototoxicity

There was no published information found on the phototoxicity in animals for most of the ingredients in the Sodium *p*-Chloro-*m*-Cresol family. There are unpublished data from RIFM in the Photoallergy (human) section, and animal data for *o*-Cymen-5-ol are described below.

*o*-Cymen-5-ol. Forty female Hartley guinea pigs, 10 per group, were used to determine the photosensitization potential of *o*-Cymen-5-ol using the adjuvant-strip test method (Osaka Kasei Co., Ltd. 1992b). Animals of the two test groups were dosed with either 1% or 2% *o*-Cymen-5-ol in a 50% ethanol solution during induction and either 0.5% and 1% or 1% and 2% *o*-Cymen-5-ol solutions for the challenge, respectively. A dose

of 2% was determined to be the maximum dose used based on a primary local dermal irritation screening assay using ultraviolet (UV) irradiation and practical concentration of use.

The positive control group was dosed with 10% 6-methylcoumarin (6-MC) in a 50% ethanol solution at both induction and challenge and the negative control group was dosed with a 50% ethanol solution. The UV-irradiation equipment (Type N-DMR; Toshiba Medical Instrument Co., Ltd.) used 12 black light bulbs (Toshiba FL40S BLB) arranged in parallel and had wavelengths in the UVA range.

For induction, a 4 × 6-cm area of the shoulder of each animal was clipped free of hair and 0.1 ml of a 1:1 w/o FCA and distilled water emulsion was injected intradermally into the four corners of the shoulder region. A mild cutaneous inflammatory reaction was induced in the shoulder region by repeated application and removal of a strip of adhesive tape. A volume of 0.1 ml of test material was then applied to the shoulder region. The test site was irradiated 30 min after application of the test solution with 10 J/cm<sup>2</sup> UV light through window glass. With the exception of the intradermal injections, this procedure was repeated once daily for 5 consecutive days.

## COSMETIC INGREDIENT REVIEW

TABLE 8

## Dermal sensitization studies

chemical	Species/ number/test	Induction/ Challenge doses	Results and comments	Reference
PCMC	20 female outbred albino guinea pigs; GPMT	<u>Induction:</u> 5% PCMC intraderm. and 10% PCMC top. <u>Challenge:</u> Occlusive patch with 1% PCMC on days 21 and 35. <u>Induction:</u> 5% of a pentachlorophenol/PCMC biocide intraderm. and 25% biocide top. <u>Challenge:</u> Finn chambers with 5% biocide on day 21, 1% PCMC or pentachlorophenol on day 35, and 0.5% biocide on day 42.	16/19 animals had reacted upon challenge on day 21; only 4/19 reacted upon challenge on day 35.  Positive upon challenge with the biocide and PCMC, but not with pentachlorophenol. Frequency of positive reactions decreased between days 21 and 42: 17/19 reacted to biocide challenge on day 21; 12/19 reacted to PCMC challenge on day 35; 6/19 reacted to biocide challenge on day 42.	Andersen and Hamann 1984a
PCMC	20 female outbred albino guinea pigs; GPMT	<u>Induction:</u> 0.2%, 1%, or 5% PCMC intraderm. and 10% PCMC top.; 0.2% PCMC intraderm. and 2% PCMC top.; or 0.2% PCMC intraderm. and 0.4% PCMC top. <u>Challenge:</u> Finn chambers with 0.1%, 0.5%, and 1% PCMC on day 21 (also on day 35 for some groups).	Positive reaction frequencies of 75%, 70%, 65%, and 84% for animals induced with 0.2%, 1%, 1%, and 5% intraderm./10% top.; 30% and 15% for animals induced with 0.2% intraderm./0.4% and 0.2% top. The 72 h, but not the 48 h, readings were significantly increased with increased challenge concentrations. Reactivity decreased from day 21 to 35.	Andersen and Hamann 1984b
PCMC	20 female outbred albino guinea pigs; GPMT	<u>Induction:</u> 1% PCMC intraderm. and 10% PCMC top.; three types of Freund's complete adjuvant (FCA) emulsions were used. <u>Challenge:</u> Finn chambers with 0.1% and 1% PCMC on day 21; patch placements were interchanged and were also placed near the abdomen.	No significant difference in response due to different FCA emulsions. After 48 h, 19, 15, and 14 animals of the three groups reacted upon challenge to 1% PCMC; after 72 h, 18, 18, and 17 reacted. Reactions were significantly weaker near the abdomen than those higher on the flank.	Andersen 1985a
PCMC	36 guinea pigs; GPMT	<u>Induction:</u> 1% PCMC intraderm. and 10% PCMC top. <u>Challenge:</u> Occlusive patches of 0.1 and 1% PCMC on day 21.	28/36 animals reacted after 48 h.	Andersen and Staberg 1985
PCMC	20 outbred albino guinea pigs; GPMT	<u>Induction:</u> 1% PCMC intraderm. and 10% PCMC top. Two groups were dosed ip with 250 mg/kg cyclophosphamide (CY) on day 18 or 32. <u>Challenge:</u> Finn chambers with 0.2%, 0.5%, and 1.0% PCMC on days 21 and 35.	Most test animals not dosed with CY prior to day 21 reacted upon challenge. The number reacting upon challenge on day 35 was reduced, especially in the group dosed with CY on day 32. Dosing with CY on day 18 reduced the number reacting to day 21, but not day 35, challenge.	Andersen 1985b

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**TABLE 8**  
Dermal sensitization studies (*Continued*)

chemical	Species/ number/test	Induction/ Challenge doses	Results and comments	Reference
PCMC	60 Stamm Pirbright White guinea pigs (30/sex); Magnusson-Kligman	<u>Induction:</u> 1 × 3 intraderm. injections (2 with PCMC and 1 with Freund's adjuvant); top. application of 1% and 25% PCMC 1 week later. <u>Challenge:</u> Cutaneous application of 12.5%, 22%, and 50% PCMC 2 week after top. induction.	The 25% solution was strongly sensitizing while the 1% solution was weakly sensitizing.	BAYER AG 1983
PCMC	35 female Stamm Pirbright White guinea pigs; Epicutaneous	<u>Induction:</u> 2 open applications of 1%, 3%, 10%, and 30% PCMC. <u>Challenge:</u> 3%, 10%, 30%, and 100% PCMC.	No sensitization reaction.	BAYER AG 1980b
PCMC	8 female outbred albino guinea pigs; OET	<u>Induction:</u> Daily open applications of 1, 3, 10, or 30% PCMC. <u>Challenge:</u> Occlusive patches with 0.5%, 1%, and 2% PCMC on day 21; open applications of 0.1%, 2%, and 5% PCMC on day 28; occlusive patches with 0.5% and 1% on day 35.	PCMC was a "doubtful" sensitizer. Doses ≥ 3% produced irritation.	Andersen and Hamann 1984b
PCMC	20 conventional female albino guinea pigs; Cumulative contact enhancement test (CCET)	<u>Induction:</u> Patches with a suspension of 5% PCMC in aqueous solution, stabilized with carbomer 941; or 5% PCMC in olive oil/acetone; propylene glycol, with or without carbomer 941 on days 0, 3, 7, and 9. <u>Challenge:</u> Finn chambers with 0.1 and 1.0% PCMC on day 21	Frequency of sensitization was vehicle related, with the aq. solution stabilized with carbomer 941 and the olive oil/acetone vehicles comparable and both higher than the propylene glycol vehicles.	Andersen et al. 1985
<i>o</i> -Cresol	24 female albino Dunkin-Hartley guinea pigs; GPMT	<u>Induction:</u> Treated with 2-methylol phenol. <u>Challenge:</u> Application with 13.1% <i>o</i> -Cresol (in ethanol).	Confluent erythema was the minimal criteria for a positive reaction. At rechallenge, 13 of 24 reacted to 2-methylol phenol and 7 of 24 guinea pigs reacted to <i>o</i> -Cresol.	Bruze 1986
<i>p</i> -Cresol	6–8 male and female Himalayan guinea pigs per group; OET	<u>Induction:</u> 4% <i>p</i> -Cresol on guinea pigs were used. Induction was done by 21 daily open applications. <u>Challenge:</u> Performed on days 21 and 35 by open application. Reactions were evaluated at 24, 48, and 72 h. No effects were reported.	No effects were reported.	RIFM 2001a

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**TABLE 8**  
Dermal sensitization studies (*Continued*)

chemical	Species/ number/test	Induction/ Challenge doses	Results and comments	Reference
<i>p</i> -Cresol	24 female albino Dunkin-Hartley guinea pigs; GPMT	<u>Induction:</u> Treated with 4-methylol phenol. <u>Challenge:</u> Application with 13.1% <i>p</i> -Cresol (in ethanol).	At rechallenge, 13 of 24 reacted to 4-methylol phenol and 4 of 24 guinea pigs reacted to <i>p</i> -Cresol.	Bruze 1986
Thymol	20 female guinea pigs; GPMT	<u>Induction:</u> Intradermal injections of FCA plus distilled water, 10% Thymol in FCA, and 10% Thymol in FCA plus distilled water. Seven days later after 10% sodium lauryl sulfate pretreatment, 0.2 ml of 10% Thymol in FCA was applied topically for 48 h under occlusion. <u>Challenge:</u> Two weeks after topical application, 0.02 ml of 5, 10, or 20% Thymol in acetone was applied topically. Reactions were graded at 24, 48, and 72 h after application, reactions were graded per Draize.	The mean response was 0.4 using 20% Thymol and 0.2 at 10% Thymol 48 h later. Weak sensitization effects with 20% Thymol were observed. Thymol was considered negative at 10% concentration under the conditions of this test.	CTFA 1997b
Thymol	6–8 male and female Himalayan guinea pigs per group; OET	<u>Induction:</u> Using 3% by 21 daily open applications to the clipped flank skin of animals. <u>Challenge:</u> Performed on days 21 and 35 by open application to the contralateral flank. Reactions were evaluated at 24, 48, and 72 h.	No effects were reported.	Klecak et al. 1977
Thymol	6–8 male and female Himalayan guinea pigs per group; Draize test	<u>Induction:</u> 0.05 ml Thymol in a 0.1% solution (in saline) was injected intradermally on day 0. On 9 alternate days, 0.1 ml of Thymol was injected for a total dose of 0.95 mg. <u>Challenge:</u> Intradermal on days 35 and 49. The evaluation criterion was the mean diameter of the papular reactions.	The Draize test was negative.	Klecak et al. 1977
Thymol	6–8 male and female Himalayan guinea pigs per group; GPMT	<u>Induction:</u> Performed via two intradermal injections (0.1 ml at 5% concentration) with and without FCA on day 0. In addition, 250 mg	No effects were observed.	Klecak et al. 1977

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**TABLE 8**  
Dermal sensitization studies (*Continued*)

chemical	Species/ number/test	Induction/ Challenge doses	Results and comments	Reference
		of Thymol (in petrolatum) at a 25% concentration was applied under occlusion on day 8 for 48 h. <u>Challenge:</u> On day 21 was by a 24-hour patch test using Thymol in petrolatum. Reactions were read at 24 and 48 h after removing the patch.		
Thymol	6–8 male and female Himalayan guinea pigs per group; FCA test	<u>Induction:</u> via intradermal injection of 0.1 ml of a 50:50 mixture of Thymol and FCA on days 0, 2, 3, 7, and 9. The total dose was 250 mg. The control animals were treated similarly with 5 × 0.05 ml of FCA alone. <u>Challenge:</u> on days 21 and 35 by a 24-h patch test using Thymol in petrolatum.	No effects were observed	Klecak et al. 1977
Thymol	Nine albino Dunkin-Hartley guinea pigs; cross-reactivity	Guinea pigs sensitized to p-methoxyphenol were tested for cross-reactivity to Thymol. Vehicle was methylethyl ketone and arachis oil.	No effects were reported.	Van Der Walle et al. 1982
<i>o</i> -Cymen-5-ol	Male Hartley guinea pigs (10/group); GPMT	<u>Induction:</u> Patches containing 1.0% <i>o</i> -Cymen-5-ol in ethyl alcohol were applied to four groups of male guinea pigs of the Hartley strain. <u>Challenge:</u> Patches consisted of either 10%, 1.0%, 0.1%, or 0.01% <i>o</i> -Cymen-5-ol in acetone.	The number of animals exhibiting skin reactions at the challenge reading were 5/10, 2/10, 1/10, and 0/10 to 10%, 1.0%, 0.1%, and 0.01% <i>o</i> -Cymen-5-ol, respectively. No skin sensitization was observed in control animals given a challenge application of ethyl alcohol. The investigator concluded that <i>o</i> -Cymen-5-ol demonstrated “very slight allergenicity.”	CTFA 1977
<i>o</i> -Cymen-5-ol	10 female Hartley guinea pigs per group; GPMT	<u>Induction:</u> Two test groups were dosed with 0.1 ml of either 1% or 2% <i>o</i> -Cymen-5-ol in a 50% ethanol solution for 48 h. <u>Challenge:</u> Animals were challenged two weeks after induction. Animals induced with 1% <i>o</i> -Cymen-5-ol were challenged with either 0.5% or 1.0% <i>o</i> -Cymen-5-ol and animals induced with 2% <i>o</i> -Cymen-5-ol were	No animals in either <i>o</i> -Cymen-5-ol test group had any dermal reactions, with the mean scores being 0/4 at both challenge sites for the two test groups. All animals dosed with DNCB had reactions, with the mean scores being 1.5 and 2.2/4 at 24 and 48 h after challenge, respectively. <i>o</i> -Cymen-5-ol was determined to have low sensitization potential under these test conditions.	Osaka Kasei Co., Ltd. 1992a

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## COSMETIC INGREDIENT REVIEW

**TABLE 8**  
Dermal sensitization studies (*Continued*)

chemical	Species/ number/test	Induction/ Challenge doses	Results and comments	Reference
		challenged with either 1 or 2% <i>o</i> -Cymen-5-ol. The challenge was for 24 h. The positive control was dosed with 0.1% dinitrochlorobenzene (DNCB) in a 50% ethanol solution at induction and challenge; the negative control group was dosed with 50% ethanol solution.		
Carvacrol	6–8 male and female Himalayan guinea pigs per group; OET	<u>Induction:</u> Was done by 21 daily open applications of 3% Carvacrol to the clipped skin on the flank. <u>Challenge:</u> Was performed on days 21 and 35 by open application to the contralateral flank. Reactions were evaluated at 24, 48, and 72 h.	The OET was positive. The minimum sensitizing concentration was 3% and the minimum eliciting concentration was 1%.	Klecak et al. 1977
Carvacrol	6–8 male and female Himalayan guinea pigs per group; Draize test	<u>Induction:</u> 0.05 ml Carvacrol in a 0.1% solution (in saline) was injected intradermally on day 0. On 9 alternate days, 0.1 ml of Carvacrol was injected for a total dose of 0.95 mg. <u>Challenge:</u> Intradermal on days 35 and 49. The evaluation criterion was the mean diameter of the papular reactions.	The Draize test was negative.	Klecak et al. 1977
Carvacrol	6–8 male and female Himalayan guinea pigs per group; GPMT	<u>Induction:</u> Performed via two intradermal injections (0.1 ml at 5% concentration) with and without FCA on day 0. In addition, 250 mg of Carvacrol (in petrolatum) at a 25% concentration was applied under occlusion on day 8 for 48 h. <u>Challenge:</u> On day 21 was by a 24-h patch test using Carvacrol in petrolatum. Reactions were read at 24 and 48 h after removing the patch.	Sensitization effects were observed in at least 2 animals; the severity of the effects were not described.	Klecak et al. 1977

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**TABLE 8**  
Dermal sensitization studies (*Continued*)

chemical	Species/ number/test	Induction/ Challenge doses	Results and comments	Reference
Carvacrol	6–8 male and female Himalayan guinea pigs per group; FCA test	<u>Induction:</u> Was performed via intradermal injection of 0.1 ml of a 50:50 mixture of Carvacrol and FCA on days 0, 2, 3, 7, and 9. The total dose was 250 mg. The control animals were treated similarly with 5 × 0.05 ml of FCA alone. <u>Challenge:</u> On days 21 and 35 by a 24-hour patch test using Carvacrol in petrolatum.	Sensitization effects were observed.	Klecak et al. 1977

Intraderm; intradermal; top.; topical.

The challenge was performed 2 weeks after induction by clipping the dorsal region free of hair and dividing the area into two 1.5 × 1.5-cm blocks, one on each side of the spinal cord. A volume of 0.1 ml of test material was applied to both test sites. Thirty minutes after application of the test material, one site was covered with aluminum foil and the other was irradiated with 10 J/cm<sup>2</sup> UV light through window glass. Dermal reactions (erythema and edema) were scored 24, 48, and 72 h after challenge. A more severe reaction to the test material at the irradiated as compared to nonirradiated site was considered indicative of a photosensitization response.

No animals in either of the *o*-Cymen-5-ol test groups had any changes in general condition during the study. One animal in the 2% *o*-Cymen-5-ol induction dose group had very slight erythema (score 1/4) after challenge with the 2% *o*-Cymen-5-ol solution at the nonirradiated site after 24 h and at both sites after 48 and 72 h; slight erythema (score 1/4) was also observed for the same animal after challenge with the 1% *o*-Cymen-5-ol solution at both sites and all observation periods. This reaction was not considered a photosensitization response because it was not more severe at the irradiated site than it was at the nonirradiated site. None of the other *o*-Cymen-5-ol–treated animals had any dermal reactions to either induction or challenge doses. Four of the positive control animals had slight erythema (score 1/4) at the irradiated site 24, 48, and 72 h after challenge. No dermal reactions were observed in the negative-control animals at either the irradiated or nonirradiated sites. *o*-Cymen-5-ol was determined to have little photosensitization potential under the test conditions used (Osaka Kasei Co., Ltd. 1992b).

**REPRODUCTIVE AND DEVELOPMENTAL TOXICITY**

*Mixed Cresols.* In a chronic inhalation study using female rats exposed to Mixed Cresols, there were some reproductive

effects observed. Female rats were exposed to Mixed Cresols at concentrations of 0.6 to 4.0 mg/m<sup>3</sup>, which caused a decrease in the number of primary follicles in the ovaries and enhanced the process of follicular atresia (degeneration and resorption of follicles before maturity). Mixed Cresols and tricresyl phosphate (three Cresol isomers attached to a common phosphate moiety at the Cresol’s hydroxy position) at a concentration of 1.0 mg/m<sup>3</sup> prolonged estral period and shortened the diestrus period. Tricresyl phosphate was noted to affect the ovaries at concentrations that did not cause general toxicity, however the specific effects were not indicated (WHO 1995).

*m-Cresol.* Akatsuka et al. (1977) examined the effects of swine prostate extract (Robaveron) on pre- and postnatal development of mice (strain ICR-SLC) offspring when administered subcutaneously. In the experiment, the drug was administered at doses of 5, 10, or 20 ml/kg/day to pregnant mice for 10 days from the 6th to 15th day of gestation. *m*-Cresol was given to the solvent group and saline to the control group in a similar manner (dose and concentration not stated). Thirty pregnant mice were used per study group.

No significant differences were observed in mean body weight gains (23.45 ± 1.06 g, control; 22.68 ± 0.66 g *m*-Cresol–treated mice) or the general behavior of the mice during pregnancy. The mean body weights, number of embryos, live fetuses, and fetal mortality did not differ significantly in any group. There were no differences between control and treated groups in the rates of external and skeletal abnormalities except for a slight increase in lumbar ribs (25 incidences in controls and 43 incidences in *m*-Cresol–treated mice), and ossification did not differ from the controls. No malformation of visceral organs was observed. No effect was seen on the postnatal development of the offspring examined (89 control and 86 *m*-Cresol–treated mice) up to week 5 post birth (Akatsuka et al. 1977).

Akatsuka et al. (1978a) examined the effects of swine prostate extract (Robaveron) administered subcutaneously to pregnant rats, using *m*-Cresol as a solvent. Pregnant rats and their fetuses were monitored until the 5th week after birth. In the experiment, the drug was administered at doses of 5, 15, or 30 ml/kg body weight/day to pregnant rats for 11 days from the 7th to 17th day of gestation. The solvent control, 0.3% *m*-Cresol, was given at 30 ml/kg/day. Saline was given to another control group. Thirty pregnant rats were used in the control group and 31 pregnant rats were used in the *m*-Cresol-treated group.

No differences were observed in mean body weights or the general behavior of the rats during pregnancy. However, the mean thymus weights in dams was decreased in those treated with *m*-Cresol as compared to the saline control (111 versus 158 mg, respectively). The mean body weights, number of embryos, live fetuses, and fetal mortality did not differ significantly in any group. There were no differences between control and treated groups in the rates of external anomalies. The offspring groups had no differences in weaning rate at 5 weeks after birth, postnatal differentiation, mean body weights, and behavior (Akatsuka et al. 1978a).

Akatsuka et al. (1978b) also reported the influence of the above treatment on postnatal development, emotion, learning and reproductive ability in the F<sub>1</sub> offspring. The F<sub>1</sub> offspring reproductive ability was unaffected by *m*-Cresol. No significant difference was noted concerning delivery rate, weaning rate, or survival rate up to 8 weeks after birth, postnatal differentiation, growth, or behavior in the F<sub>1</sub> generation. The F<sub>2</sub> generation, obtained from F<sub>1</sub> mating, had no differences in the number of embryos, live fetuses, and mean body weights. On external examination, 37/46 animals in the Robaveron treatment groups, 21/21 in the solvent group, and 7/19 in the control group were found to have hematomas. The authors stated there were no differences between the groups.

Akatsuka et al. (1978c) examined the effects of swine prostate extract (Robaveron) when administered subcutaneously to pregnant rabbits using *m*-Cresol as the solvent. The drug was administered at doses of 1, 5, or 10 ml/kg/day to pregnant rabbits from the 6th to 18th day of gestation. The solvent control, 0.3% *m*-Cresol, was given at a dose of 10 ml/kg/day. Saline was given to another control group. Eleven pregnant rabbits were used per study group.

No differences were observed in mean body weights, water consumption, or the general behavior of the rabbits during pregnancy. However, there was decreased food consumption after day 14 of gestation in the *m*-Cresol-treated group. No differences were observed in the number of resorptions, dead fetuses, live fetuses, or external anomalies. However, the rabbits in the *m*-Cresol group did experience a decrease in body weights and the number of implantations as compared to the saline control. Skeletal development and variation was considered comparable to the controls (Akatsuka et al. 1978c).

Testicular and ovarian weights were quantified at the end of the Dietz and Mulligan (1988b) subchronic study using rats

orally given *m*-Cresol by gavage. There were no effects on testicular or ovarian weights at any dose tested up to 450 mg/kg/day for 13 weeks.

The Bushy Run Research Center (BRRC 1988a) evaluated the developmental toxicity of *m*-Cresol in Sprague-Dawley rats. Timed-pregnant rats were dosed by gavage with 0, 30, 175, or 450 mg *m*-Cresol/kg/day in corn oil on gestational days (GDs) 6 through 15. There were 25 rats per test group and 50 rats used as controls. Severe maternal toxicity was evident at 450 mg/kg/day. Maternal toxicity effects included reduced food consumption, decreased body weight gains, and clinical signs such as audible respiration, hypoactivity, ataxia, and tremors. *m*-Cresol had no effect on the developing embryo at any dose tested. For *m*-Cresol, the NOEL for maternal toxicity was 175 mg/kg/day. The NOEL for developmental toxicity was at least 450 mg/kg/day. There were no reported effects on reproductive parameters (such as number of ovarian corpora lutea, number of implantation sites, number of viable fetuses).

BRRC (1988b) also evaluated the developmental toxicity of *m*-Cresol in pregnant New Zealand white rabbits. Timed-pregnant rabbits were dosed orally with 5.0, 50, or 100 mg/kg/day *m*-Cresol in corn oil on (GDs) 6 through 18. There were 14 rabbits per test group and 28 mated females in the concurrent vehicle control group (corn oil only). The dose volume of 1.0 ml/kg was based on the body weights of each female on GD 6. The rabbits were killed on GD 29 and evaluated for body weights, liver and gravid uterine weight, number of corpora lutea, and number and status of implantation sites. All live fetuses were counted, weighed, and examined for external (and visceral) malformations. Fetuses were also examined for craniofacial and skeletal malformations.

*m*-Cresol at the doses administered did not cause treatment-related deaths, abortions, or early deliveries; 12 to 14 litters per group were examined for *m*-Cresol. For the concurrent vehicle control, 23 litters were examined. There were no statistically significant changes in periodic maternal body weights or weight gain for rabbits treated with *m*-Cresol. Clinical signs of maternal toxicity (audible respiration and ocular discharge) were observed at 50 and 100 mg/kg/day. There were no treatment-related effects on food consumption and no treatment-related lesions or changes in maternal organs were noted at necropsy. Gestational parameters were not affected by *m*-Cresol; no changes in total, nonlive, or live embryos per litter or fetal body weights per litter were observed. There were no significant differences among *m*-Cresol-treated groups in the incidence of individual malformations, malformations by category, or of total malformations.

There was one visceral variation (increased incidence of pale gallbladder at 5.0 mg/kg/day) and one skeletal variation (decreased incidence of rudimentary thirteenth rib on the first lumbar arch at 50 mg/kg/day) that were significantly different relative to controls. There were no differences among groups in the incidence of individual external variations, variations by category, or of total variations. No embryotoxicity or teratogenicity were observed at any dose of *m*-Cresol employed. For

*m*-Cresol, the NOEL for maternal toxicity was 5.0 mg/kg/day and the NOEL for developmental toxicity was at least 100 mg/kg/day (BRRRC 1988b).

Gulati et al. (1988b) reported the reproductive effect of feeding F344 rats and B6C3F<sub>1</sub> mice a mixture of *m*-Cresol/*p*-Cresol for 90 days. Mice received *m*-Cresol/*p*-Cresol at concentrations of 0%, 0.625%, 0.25%, or 1.0% and rats received concentrations of 0%, 0.188%, 0.75%, or 3.0%. Vaginal smears were taken on day 90. There was a dose-related increase in the average length of the estrous cycle in rats. *m*-Cresol/*p*-Cresol had no effect on the estrous cycle of mice. Average estrous cycle length in rats increased significantly from 4.50 days in control rats to 5.10 days at the 0.75% dose level and 5.13 days in the 3.0% dose group. Terminal body weights were decreased 6% in female mice in the 1.0% dose group and almost 17% in female rats in the 3.0% dose group.

In male mice and rats, there was equivocal evidence that *m*-Cresol/*p*-Cresol caused a decrease in sperm motility, sperm count, testicular weights, or epididymal weights. Male terminal body weights were decreased 16% in the high-dose rats, but were not decreased in any of the mice dose groups (Gulati et al. 1988b).

The BRRRC (1989c) conducted a two-generation reproduction study on rats using *m*-Cresol. Groups of 25 female and 25 male Sprague-Dawley CD rats were given 0, 30, 175, or 450 mg *m*-Cresol/kg/day by gavage in corn oil for 10 weeks prior to breeding. Dosing of females continued through the 3-week mating period, gestation, and lactation. After weaning, pups were given the same doses as their parents for 11 weeks. F<sub>1</sub> females were dosed through the 3-week mating period, gestation, and lactation just as the F<sub>0</sub> females had been. All F<sub>2</sub> pups were killed at weaning. In the F<sub>0</sub> rats, toxic effects were mostly limited to the 450 mg/kg dose group and included death, reduced body weight gains, and clinical signs such as hypoactivity, ataxia, twitches, tremors, prostration, rapid and labored respiration, urine stains, and perioral wetness.

In the F<sub>1</sub> rats, some clinical signs of toxicity were seen in the 175 mg/kg group; however, no effects on the reproductive function or on the morphology of reproductive tissues were detected even at doses that produced parental toxicity. Decreased numbers of spermatazoa and atrophy of seminal vesicles were observed in some F<sub>0</sub> males treated with 450 mg *m*-Cresol/kg, which were attributed to postmortem changes or nonspecific stress and were not considered treatment related. *m*-Cresol was a more potent developmental toxicant among the Cresol isomers in the two-generation study. *m*-Cresol had effects on body weights of offspring at the low dose of 30 mg/kg/day and reduced pup survival during lactation at the high dose of 450 mg/kg/day.

Parental toxicity was reported at the low dose of 30 mg/kg/day, but developmental effects could occur at doses lower than those producing parental toxicity. Therefore, this study is inconclusive regarding developmental toxicity of *m*-Cresol (BRRRC 1989c).

The NTP (1992a) exposed mice to a mixture of *m*-Cresol/*p*-Cresol at 0, 300, 1000, 3000, 10,000, or 30,000 ppm in feed for 28 days, which were then examined for any effects upon the reproductive organs. Uterine and ovarian hypoplasia was observed in one of five B6C3F<sub>1</sub> mice in the high dose group. Testis weights, relative to body weights, were increased but no pathologic changes were noted in male mice.

The effect on the reproductive organs of mice treated with 0, 625, 1250, 2500, 5000, or 10,000 ppm *m*-Cresol/*p*-Cresol in feed was evaluated in a 13-week study. No treatment-related gross or microscopic pathology was noted (NTP 1992a).

NTP (1992b) evaluated the reproductive toxicity of a mixture of *m*-Cresol and *p*-Cresol (59%, 41%) using CD-1 Swiss mice under the Continuous Breeding Protocol. Mice received the Cresol mixture in feed at concentrations of 0.25%, 1.0%, and 1.5% for 14 weeks with exposure amounts ranging from a mean of 362 to 1682 mg/kg/day. Crossover breeding of control and 1.5% Cresol mixture F<sub>0</sub> treated mice were performed.

The Cresol mixture at 1.5% in the diet significantly reduced litter size (only 80% of control values), adjusted pup weight (only 95% of controls), and increased cumulative days to litter in the 2nd to 5th litter by 3 to 4 days.

Crossover breeding of control and 1.5% Cresol mixture F<sub>0</sub> treated mice produced decreased adjusted live pup weight of litters with a treated parent of either sex.

At necropsy, high dose F<sub>0</sub> males had decreased body weights (only 90% of control values) and decreased relative seminal vesicle weights. In males, relative kidney and liver weights increased at 1.0% and 1.5% concentrations. In females, relative liver weights increased at all doses, whereas body weights decreased 6% at a dose of 1.5%. Pre- and postweaning growth and survival were affected adversely at 1.0% and 1.5%. At 1.5% Cresol mixture, there was no effect on reproductive competence; however, F<sub>1</sub> postnatal growth and survival and F<sub>2</sub> pup weight decreased. Decreased body weights (in both sexes) and prostate weights at 1.0% and 1.5% were noted at necropsy. Relative liver and kidney weights were increased at all doses for both sexes.

The authors concluded that the *m*-Cresol/*p*-Cresol mixture at 1.0% caused minimal adult reproductive and significant postnatal toxicity in the presence of systemic toxicity (NTP 1992b).

Chapin et al. (1998) found that necropsy data from general toxicity studies can provide a preliminary indication of the likely reproductive toxicity of the compound. *m*-Cresol/*p*-Cresol was assessed using data from the above NTP study. The researchers considered data on sperm motility and vaginal cytology evaluations (SMVCEs), which were performed at the end of 90-day toxicity tests and Reproductive Assessment by Continuous Breeding (RACB) design. *m*-Cresol/*p*-Cresol was considered a possible reproductive toxicant, but the data was only suggestive in both tests.

*o*-Cresol. BRRRC (1988a) evaluated the developmental toxicity of *o*-Cresol in Sprague-Dawley rats. Timed-pregnant rats were dosed by gavage with 0, 30, 175, or 450 mg *o*-Cresol/kg/day

in corn oil on gestational GDs 6 through 15. There were 25 mated females per test group and 50 mated females used as controls.

Maternal toxicity was evident at 450 mg/kg/day. Effects included death (4 dams at 450 mg/kg/day), reduced feed consumption, decreased body weight gains, and clinical signs such as audible respiration, hypoactivity, ataxia, and tremors. *o*-Cresol caused mild fetotoxic effects (increased incidences of dilated lateral ventricles in the brain) at 450 mg/kg/day, which may have been secondary to maternal toxicity. For *o*-Cresol, the NOEL for maternal toxicity was 175 mg/kg/day. The NOEL for developmental toxicity was 175 mg/kg/day. There were no reported effects on the reproductive parameters (such as number of ovarian corpora lutea, number of implantation sites, number of viable fetuses) in rats, even at maternally toxic doses (BRRC 1988a).

The developmental toxicity of *o*-Cresol was evaluated in New Zealand white rabbits (BRRC 1988b). Timed-pregnant rabbits were dosed with 5.0, 50, or 100 mg/kg/day *o*-Cresol in corn oil on GDs 6 through 18. There were 14 mated females per test group and 28 mated females in the concurrent vehicle control group (corn oil only). The dose volume of 1.0 ml/kg was based on the body weights of each female on GD 6. The rabbits were killed on GD 29 and evaluated for body weights, liver and gravid uterine weights, number of corpora lutea, and number and status of implantation sites. All live fetuses were counted, weighed, and examined for external and visceral malformations and variations. Fetuses were also examined for craniofacial and skeletal malformations and variations.

*o*-Cresol at the doses administered did not cause treatment-related deaths, abortions, or early deliveries; 13 to 14 litters per groups were examined for *o*-Cresol. For the concurrent vehicle control, 23 litters were examined. There were no statistically significant changes in periodic maternal body weights or weight gains at any time point. Clinical signs of toxicity, including ocular discharge and audible respiration, were observed at 50 and 100 mg/kg/day, respectively. Hypoactivity was noted in rabbits dosed with 50 or 100 mg/kg/day. Food consumption was increased at 50 mg/kg/day (GDs 9–10, 26–27, and 27–28 only) and 100 mg/kg/day (GDs 9–10 only).

There were no treatment-related lesions or changes in maternal organ weights at necropsy. Gestational parameters were not affected by *o*-Cresol; no changes in total, nonlive, or live embryos per litter or fetal body weights per litter were observed. For *o*-Cresol there were two variations, ecchymosis (subepidermal hematoma) on the head and poorly ossified sternebra number 6, which were significantly increased at 100 mg/kg/day. All other incidences that were statistically significant in one or more Cresol-exposed groups relative to controls were not dose related and/or did not indicate developmental toxicity. There were no significant changes in the incidence of any variations by category or of total variations. There were no significant changes in the incidence of individual malformations, malformations by category, or of total malformations.

There were three skeletal variations that were significantly different. Two of these were extra 13th ribs on the first lumbar

arch at 50 mg/kg/day and some phalanges of the forelimb were poorly ossified at 100 mg/kg/day. There were no differences among groups in the incidence of individual external variations, variations by category or of total variations. No embryotoxicity or teratogenicity were observed at any dose of *o*-Cresol employed. For *o*-Cresol, the NOEL for maternal toxicity was 5.0 mg/kg/day and the NOEL for developmental toxicity was 50 mg/kg/day (BRRC 1988b).

Testicular and ovarian weights were measured at the end of the Dietz and Mulligan (1988a) subchronic study using *o*-Cresol. There were no effects on testicular or ovarian weights at any dose tested up to 600 mg/kg/day *o*-Cresol for 13 weeks.

Gulati et al. (1988a) evaluated the reproductive effect of feeding F344 rats and B6C3F<sub>1</sub> mice *o*-Cresol for 90 days. Mice and rats were fed *o*-Cresol at concentrations of 0%, 0.125%, 0.5%, or 2.0% and of 0%, 0.188%, 0.75%, or 3.0%, respectively. Average estrous cycle length in mice increased significantly from 4.2 days in control mice to 4.8 days at the 2.0% dose level. There was no effect on the estrous cycle in female rats fed *o*-Cresol at any dose level administered. Terminal body weights were decreased by approximately 15% in female high dose mice and rats fed *o*-Cresol. Male mice and rats were tested at the same doses as females. There was a dose-related trend of decreased caudal epididymal weights in mice. At 0.5% *o*-Cresol, right epididymal weights and caudal weights were significantly decreased at the 2.0% dose level. In mice and rats, there was no effect on sperm motility, sperm count, or testicular weight per gram of caudal tissue. Terminal body weights were decreased 16% in the high dose male rats and 15% in high dose male mice.

The BRRC (1989a) conducted a two-generation reproduction study on rats using *o*-Cresol. Groups of 25 female and 25 male Sprague-Dawley CD rats were given 0, 30, 175, or 450 mg *o*-Cresol/kg/day by gavage in corn oil for 10 weeks prior to breeding. Dosing of females continued through a 3-week mating period, gestation, and lactation. After weaning, pups were given the same dose as their parents for 11 weeks. F<sub>1</sub> females were dosed through a 3-week mating period, gestation, and lactation just as the F<sub>0</sub> females had been. All F<sub>2</sub> pups were killed at weaning. In the F<sub>0</sub> rats, toxic effects were mostly limited to the 450 mg/kg dose group and included death, reduced body weight gains, and clinical signs such as hypoactivity, ataxia, twitches, tremors, prostration, rapid and labored respiration, urine stains, and perioral wetness. In the F<sub>1</sub> rats, some clinical signs of toxicity were seen in the 175 mg/kg group; however, effects on the reproductive function or the morphology of reproductive tissues was not detected even at doses that produced parental toxicity. Rats treated with 450 mg/kg/day of *o*-Cresol had overt toxicity and produced F<sub>1</sub> offspring that had reduced body weights up to 4 weeks after birth. The NOEL for offspring in this study was 175 mg/kg/day.

NTP (1992a) performed 28-day toxicity studies to evaluate dietary exposure of *o*-Cresol to B6C3F<sub>1</sub> mice. Male and female mice were exposed to *o*-Cresol at 0, 300, 1000, 3000, 10,000, or 30,000 ppm in feed for 28 days. Female mice exposed to

30,000 ppm *o*-Cresol for 28 days had microscopic uterine and ovarian lesions. The ovaries had mild or minimal decreases in the size of interstitial, follicular, and/or luteal cells. Hypoplastic changes in the uterine endometrium and myometrium were evident. Endometrial glands were smaller and more dense; and the cytoplasm of the endometrial stromal cells and the myometrial smooth muscle cells was sparse. Similar changes, but less severe, were seen in female mice exposed at 10,000 ppm *o*-Cresol. No uterine or ovarian changes were seen in mice exposed to 300, 1000, or 3000 ppm *o*-Cresol.

The NTP also evaluated the effects upon the reproductive organs of mice treated with either 0, 1250, 5000, 10,000 or 20,000 ppm *o*-Cresol in feed in a 13-week study. Right testis weights relative to body weights were increased when B6C3F<sub>1</sub> mice were exposed to 20,000 ppm *o*-Cresol. No other treatment-related gross or microscopic pathology was noted (NTP 1992a).

NTP (1992c) assessed the reproductive toxicity of *o*-Cresol using CD-1 Swiss mice under the Continuous Breeding Protocol. Forty mice per sex served as controls. Twenty mice per sex per group received *o*-Cresol in feed at 66 mg/kg/day (0.05%), 264 mg/kg/day (0.2%), and 660 mg/kg/day (0.5%) for 14 weeks. Males and females from the same dose group were paired at the beginning of week 2 and were housed together for 13 additional weeks. Each male was then housed separately. Females remained with their litters until postnatal day 21. Dosed feed was continually supplied. Data on clinical signs, parental body weights, fertility (number producing a litter/number of breeding pairs), litters per pair, live pups per litter, proportion of pups born alive, sex of live pups, pup body weights (0 to 24 h after birth), and feed and water consumption. Offspring from control and high dose mice (20 mice per sex, per group) were evaluated. Mice received the same doses as their parents, the calculated exposure was 773 and 1128 mg/kg/day for adult F<sub>1</sub> generation males and females, respectively. Once the offspring reached sexual maturity (74 ± 10 days), males and females from the same treatment groups were paired for 7 days or until a vaginal copulatory plug was found, whichever was less time. Mice were then separated. After delivery of all the litters, all animals were killed and necropsied.

*o*-Cresol at doses up to 0.5% (1230 mg/kg/day) did not result in reproductive or general toxicity in either generation. Reproductive competence, including initial fertility, mean number of litters per pair, live litter size, the proportion of pups born alive, or adjusted live pup weight, were not affected. A small, significant increase in cumulative days (2 to 3 days) to litter was observed in all treated groups, but this increase was not dose related. After 16 weeks, F<sub>0</sub> body weights and feed and water consumption were unaffected. There was no effect on body or liver, kidney, or testis weights at necropsy, except for a decrease in absolute kidney weights in high dose females. Female terminal body weights in F<sub>1</sub> mice was slightly reduced, but there was no effect on organ weights, vaginal cytology, or liver, kidney, or ovarian histopathology. There was no effect on male body weights, liver, epididymis, kidney, prostate, semi-

nal vesicle, testis weights, sperm parameters, or histopathology. The *o*-Cresol NOAEL was 0.2% for both reproductive and general toxicity in both generations. The authors concluded that the data indicated that *o*-Cresol is not a reproductive toxicant to F<sub>0</sub> or F<sub>1</sub> mice under the conditions of this study; i.e., doses up to 1230 mg/kg/day (NTP 1992c).

Chapin et al. (1998) found that reproductive-system necropsy data from general toxicity studies can provide a preliminary indication of the likely reproductive toxicity of the compound. *o*-Cresol was assessed using data from the NTP study (1992c). The researchers considered data from SMVCEs which were performed at the end of 90-day toxicity tests and RACB design. *o*-Cresol's reproductive toxicity was not well predicted because it was considered a reproductive toxicant in SMVCEs, but was not classified as a reproductive toxicant in the RACB design.

*p*-Cresol. The BRRRC (1988a) evaluated the developmental toxicity of *p*-Cresol in Sprague-Dawley rats. Timed-pregnant rats were dosed by gavage with 0, 30, 175, or 450 mg *p*-Cresol/kg/day in corn oil on GDs 6 through 15. There were 25 animals per test group and 50 animals used as controls. Maternal toxicity was evident at 450 mg/kg/day. Effects included death (3 dams at 450 mg/kg/day), decreased feed consumption, decreased body weight gains, and clinical signs such as audible respiration, hypoactivity, ataxia, and tremors. *p*-Cresol caused mild fetotoxic effects (such as minor skeletal variations) at 450 mg/kg/day, which may have been secondary to maternal toxicity. For *p*-Cresol, the NOEL for maternal toxicity was 175 mg/kg/day. The NOEL for developmental toxicity was 175 mg/kg/day. There were no reported effects on the reproductive parameters (such as number of ovarian corpora lutea, number of implantation sites, number of viable fetuses) in rats, even at maternally toxic doses.

The BRRRC (1988b) evaluated the developmental toxicity of *p*-Cresol in New Zealand white rabbits. Timed-pregnant rabbits were dosed with 5.0, 50, or 100 mg/kg/day of *p*-Cresol in corn oil on GDs 6 through 18. There were 14 animals per test group and 28 animals in the concurrent vehicle-control group (corn oil only). The dose volume of 1.0 ml/kg was based on the body weights of each female on GD 6. The rabbits were killed on GD 29 and evaluated for body weights, liver and gravid uterine weights, number of corpora lutea, and number and status of implantation sites. All live fetuses were counted, weighed, and examined for external (and visceral) malformations and variations. Fetuses were also examined for craniofacial and skeletal malformations and variations.

For *p*-Cresol, five rabbits (35.7%) in the 100 mg/kg/day group and two rabbits (14.3%) in the 50 mg/kg/day group died.

There were no treatment-related abortions, early deliveries, or resorbed litters; 6 to 11 litters per groups were examined for *p*-Cresol. For the concurrent vehicle control, 23 litters were examined. There were no statistically significant changes in periodic maternal body weights or weight gains at any time point for rabbits treated with *p*-Cresol. The clinical signs of toxicity, ocular discharge, and audible respiration were observed at 50 and 100 mg/kg/day, respectively. Hypoactivity, gasping,

cyanosis, and audible and labored respiration were noted in rabbits dosed with 50 or 100 mg/kg/day of *p*-Cresol. There were no treatment-related effects on food consumption at any time throughout gestation.

There were no treatment-related lesions or changes in maternal organ weights noted at necropsy. Gestational parameters were not affected by *p*-Cresol; no changes in total, nonlive, or live embryos per litter or fetal body weights per litter were observed. However, the sex ratio (more males) was significantly increased at 50 mg/kg/day. This was attributed to biological variability because it was neither dose related nor accompanied by change in litter size, percent live fetuses per litter, or number of nonviable embryos. There were no significant changes in the incidence of individual malformations, malformations by category, or of total malformations. There were no significant differences among groups in the incidence of individual external variations, variations by category, or of total variations. Embryotoxicity or teratogenicity were not observed at any dose of *p*-Cresol employed. For *p*-Cresol, the NOEL for maternal toxicity was 5.0 mg/kg/day and the NOEL for developmental toxicity was at least 100 mg/kg/day (BRRC 1988b).

Testicular and ovarian weights were quantified at the end of the Dietz and Mulligan (1988c) subchronic study using *p*-Cresol. After 13 weeks, there were no effects on testicular or ovarian weights at any dose tested except for the highest dose tested (600 mg/kg/day). The right ovary of rats treated with *p*-Cresol for 13 weeks at 600 mg/kg/day weighed significantly less than the right ovaries of control rats. The left ovary of *p*-Cresol rats from the high dose group had a normal weight. No abnormal histopathology was noted in the right ovaries. The authors noted Cresol doses that can be lethal to rats generally do not affect reproductive organ weights.

The BRRC (1989b) also conducted a two-generation reproduction study on rats using *p*-Cresol. Groups of 25 female and 25 male Sprague-Dawley CD rats were given 0, 30, 175, or 450 mg *p*-Cresol/kg/day by gavage in corn oil for 10 weeks prior to breeding. Dosing of females continued through a 3-week mating period, gestation, and lactation. After weaning, pups received the same dose as their parents for 11 weeks. F<sub>1</sub> females were dosed through a 3-week mating period, gestation, and lactation just as the F<sub>0</sub> females had been. All F<sub>2</sub> pups were killed at weaning.

In the F<sub>0</sub> rats, toxic effects were mostly limited to the 450 mg/kg dose group and included death, reduced body weight gains, and clinical signs such as hypoactivity, ataxia, twitches, tremors, prostration, rapid and labored respiration, urine stains, and perioral wetness. In the F<sub>1</sub> rats, some clinical signs of toxicity were seen in the 175 mg/kg group; however, effects on reproductive function or the morphology of reproductive tissues was not detected even at doses that produced parental toxicity. Decreased numbers of spermatozoa in some F<sub>1</sub> males treated with 450 mg/kg *p*-Cresol was not considered treatment related. Rats treated with 450 mg/kg/day of *p*-Cresol had overt toxicity in the parents and produced F<sub>1</sub> offspring that had reduced body

weights four weeks after birth. The NOEL for offspring in this study was 175 mg/kg/day (BRRC 1989b).

Kavlock (1990) evaluated the maternal and developmental toxicity of *p*-Cresol using Sprague-Dawley rats. Groups of 13 to 17 timed-pregnant rats (weighing 180 to 200 g) received 0, 100, 333, 667, or 1000 mg/kg of *p*-Cresol in a vehicle of water, Tween 20, propylene glycol, and ethanol (in a ratio of 4:4:1:1) by intubation on day 11 of gestation. Intubation volume was 10 ml/kg body weight. The control group of 15 to 20 females received the vehicle only. All animals received feed and water available ad libitum. Females were weighed on days 10, 11, 12, 14, 17, and 21 of gestation and were killed at weaning of the litter and the number of implantation scars in the uterus were noted.

Only one female in highest dose group died. There was a significant weight loss in the female rats 24 h after being dosed with *p*-Cresol; these changes were observed in the 333, 667, and 1000 mg/kg groups ( $-13 \pm 3$ ,  $-19 \pm 3$ ,  $-28 \pm 3$  g, respectively). After 72 h, all except the high dose group, seemed to recover the weight loss. There were no significant differences between the groups in terms of number of pregnant rats, litter size at postnatal days 1 and 6, perinatal loss, pup weight at postnatal day 1 and 6, and litter biomass at postnatal day 6. The authors qualitatively summarized *p*-Cresol as an active maternal toxicant but as an inactive developmental toxicant; it is likely that the mechanisms of developmental and maternal toxicity are different (Kavlock 1990).

Oglesby et al. (1992) reported a follow-up study that evaluated the embryotoxicity of *p*-Cresol on mid-gestation rat embryos in vitro. The information was used in conjunction with its structure and activity to correlate with in vivo data. Female Sprague-Dawley rats were used and food and water were available ad libitum. Embryos were explanted and hepatocytes were obtained from pregnant Sprague-Dawley rats on gestation day 10. Embryos alone or in coculture with hepatocytes were exposed to *p*-Cresol in vehicle at concentrations of 0, 15, 25, 50, and 75  $\mu$ g/ml. At 42 h in culture, embryos with a detectable heartbeat were scored as viable and evaluated for growth and morphological abnormalities.

*p*-Cresol in the absence of hepatocytes caused no significant increases in the incidences of fore or hind limb bud absence, hyperplasia of 1st arch, bifurcated tails, or total tail defects at 15 or 25  $\mu$ g/ml. However, at 50  $\mu$ g/ml, 42% of viable embryos had hind limb bud absence or total tail defects. At 75  $\mu$ g/ml, 88% of viable embryos had hind limb bud absence and 50% had total tail defects. Three growth end points (somite number, crown-rump length, and DNA content) were compared to concurrent controls. At a concentration of 15  $\mu$ g/ml of *p*-Cresol, only somite number was decreased significantly from 35.1 to 32.2. At a concentration of 25  $\mu$ g/ml of *p*-Cresol, there was a significant decrease in somite number (from 35.1 to 29.6) and DNA content (from 47.5 to 41.1  $\mu$ g). At a concentration of 50  $\mu$ g/ml of *p*-Cresol, there was a significant decrease in somite number (from 35.1 to 27.16), crown-rump length (from 4.8 to 4.2 mm), and DNA content

(from 47.5 to 30.3  $\mu\text{g}$ ). At a concentration of 75  $\mu\text{g}/\text{ml}$  of *p*-Cresol, there was a significant decrease in somite number (from 35.1 to 24.3), crown-rump length (from 4.8 to 3.7 mm), and DNA content (from 47.5 to 25.1  $\mu\text{g}$ ).

The embryotoxicity of *p*-Cresol was greatly diminished by hepatocytes, presumably due to phase II conjugation reactions. *p*-Cresol in the presence of hepatocytes resulted in no incidences of fore or hind limb bud absence, hyperplasia of 1st arch, or bifurcated tails at all concentrations tested; however, there was a slight but insignificant increase in total tail defects at 50 and 75  $\mu\text{g}/\text{ml}$  (seen in 8% and 11% of the viable embryos). In the presence of hepatocytes, 75  $\mu\text{g}/\text{ml}$  *p*-Cresol showed a significant decrease in somite number (from 35.2 to 29.3), crown-rump length (from 5.0 to 4.2 mm), and DNA content (54.8 to 33.3  $\mu\text{g}$ ). There was also a significant decrease in somite number at 25  $\mu\text{g}/\text{ml}$  of *p*-Cresol (from 35.2 to 33.9), *p*-Cresol at 15 and 50  $\mu\text{g}/\text{ml}$  had no significant effect on somite number as compared to controls (Oglesby et al. 1992).

**Thymol.** Kar (1960) conducted a study in which the ability of Thymol to inhibit the response of the genital organs of immature female rats to exogenous gonadotropic hormone was evaluated. Each rat received 1 mg Thymol (in propylene glycol vehicle) subcutaneously daily for 4 days and a single administration of 40 IU hormone (gonadotropin) on day 2. There were six female rats per group and they were killed on day 5; the ovary and uterus were weighed. There was no significant effect on the response of the ovaries to hormone stimulation; however, there was marked increase (+50.06%) in uterine weights as compared to rats treated with gonadotropin alone.

Verrett et al. (1980) injected Thymol into chicken embryos via the air cell and the yolk at preincubation (0 h) and 96 h. At least 100 embryos per dose level were treated at a minimum of five dose amounts. Appropriate groups of vehicle controls and untreated controls were included. LD<sub>50</sub> values were determined.

Thymol produced teratogenic effects in the developing chicken embryo. The LD<sub>50</sub> values in the air cell were 1.16 and 0.06 mg/egg at 0 and 96 h, respectively. The yolk sac LD<sub>50</sub> values were 4.66 and 1.02 mg/egg at 0 and 96 h, respectively. The highest level tested was 25.0 mg/egg. At preincubation, Thymol caused 0% to 36.13% and 1.73% to 15.65% of embryos to develop abnormally when treated via the air cell and the yolk sac, respectively. At 96 h, Thymol caused 0% to 13.57% and 0.90% to 6.36% of embryos to develop abnormally when treated via the air cell and the yolk sac, respectively. The incidences of abnormalities were statistically significant compared to controls for air cell treatment, but not for yolk treatment (Verrett et al. 1980).

## GENOTOXICITY

Genotoxicity studies of Mixed Cresols, *m*-Cresol, *o*-Cresol, *p*-Cresol, Thymol, and Carvacrol in *Salmonella typhimurium* are summarized in Table 9. They uniformly demonstrate no effect.

Genotoxicity studies of Cresol ingredients in other test systems are described below.

**PCMC.** A modified SOS chromotest was performed using *Escherichia coli* strain PQ37 to measure the genotoxic potential of PCMC (Malaveille et al. 1991). PCMC, at concentrations of 0.1 to 4 mM, induced SOS-DNA repair synthesis without metabolic activation. The addition of a metabolic activation system decreased the genotoxicity of PCMC. Also, the addition of a radical scavenger, Trolox C (a hydrosoluble form of vitamin E), inhibited the genotoxicity of PCMC at 0.3 mM.

**Mixed Cresols.** Litton Bionetics, Inc., tested Mixed Cresols in a series of assays. Litton Bionetics, Inc. (1980a) treated L5178Y/TK<sup>+/-</sup> mouse lymphoma cells with 0.977 to 750,000 nL/ml of Mixed Cresols without metabolic activation for 4 h. Negative control cells were treated with dimethylsulfoxide alone. Following cell treatment and rinsing, cultures were placed in growth medium for 2 to 3 days to allow for growth and expression of the induced TK<sup>-/-</sup> phenotype. Cell counts were done daily and appropriate dilutions were made to maximize growth. Following a 10-day incubation period, the colonies were sized and counted. L5178Y/TK<sup>+/-</sup> cells were also treated with 0.488 to 31,300 nL/ml of Mixed Cresols with metabolic activation. For metabolic activation, the only difference in the protocol was the addition of the S9 fraction of rat liver homogenate and necessary cofactors during the 4-h treatment period. The components of the activation system in the cell suspension assays were NADP (sodium salt), isocitric acid, and S9. Ethylmethane sulfonate was the positive control used for the nonactivation studies and dimethylnitrosamine was the positive control used for the metabolic activation studies.

Mixed Cresols induced dose-related increases in the mutation frequency at the TK locus in the L5178Y mouse lymphoma cells in the presence of rat liver S9 microsomal activation. However, without activation, Mixed Cresols was suggestive of only weak mutagenic activity because concentrations up to 750 nL/ml were highly toxic. With S9 activation, high toxicity occurred at 31.3 nL/ml and mutagenesis was detectable from 3.9 to 7.8 nL/ml. Four to eightfold increases in mutation frequency were observed at 31.3 nL/ml (Litton Bionetics, Inc. 1980a).

Litton Bionetics, Inc. (1980a) also tested Mixed Cresols in the in vitro transformation of BALB/c 3T3 cells assay with activation by primary rat hepatocytes. First, a cytotoxicity assay was performed to determine the effect of various concentrations of Mixed Cresols on the colony forming ability of 3T3 cells after 48 h of exposure in the presence of rat hepatocytes. A series of 25-cm<sup>2</sup> flasks were each seeded with  $1 \times 10^3$  primary rat hepatocytes/flask and incubated for 24 h. Afterward,  $1 \times 10^4$  3T3 cells were seeded into each culture and after a 24-h cocultivation period, 15 flasks were treated with each of the five preselected concentrations of Mixed Cresols (0.01, 0.10, 1.00, 4.00, and 8.00 nL/ml).

Fifteen positive (dimethylnitrosamine) and 15 negative (DMSO) controls were also prepared. The flasks were incubated for 2 days and then rinsed. Incubation then continued for

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**TABLE 9**  
Genotoxicity of Cresols and Isopropyl Cresols in *Salmonella typhimurium*

Ingredient	Strains	Protocol and dose	Results	Reference
PCMC	TA1535, TA100, TA1537, and TA98	Ames test in the presence of metabolic activation. Two positive controls, endoxan and tryptaflavin, were used. PCMC was used at $\leq 500 \mu\text{g}/\text{plate}$ .	Negative.	BAYER AG 1980c
PCMC	TA100	PCMC was tested at $10^{-1}$ – $10^3 \mu\text{g}/\text{plate}$ .	Negative.	Rapson et al. 1980
PCMC	TA100, TA1535, TA1537, and TA98	Doses of 1.28, 6.4, 32, 160, and 800 $\mu\text{g}$ PCMC per plate were tested with and without metabolic activation; each dose was tested in five parallel plates and two runs were performed. The vehicle, DMSO, served as the negative control. Without metabolic activation, the positive controls were sodium azide (TA1535 and TA1000) and 2-nitrofluorene (TA1537 and TA98); with metabolic activation, 2-antramine served as the positive control for all strains.	Negative. The number of His <sup>+</sup> revertants observed per plate using 1.28–32 $\mu\text{g}/\text{plate}$ PCMC was similar to the value for the negative control. However, concentrations of 160 and 800 $\mu\text{g}/\text{plate}$ generally produced an increasing toxic effect on the tester strains; metabolic activation seemed to lessen this effect.	Madsen et al. 1986
PCMC	TA100, TA1535, TA1537, TA97, and TA98	PCMC in DMSO was used at 3.3 to 666.0 $\mu\text{g}/\text{plate}$ . The vehicle was used as a negative control. Without metabolic activation, the positive controls were sodium azide (TA100 and TA1535), 9-aminoacridine (TA1537 and TA97), and 4-nitro-o-phenylenediamine (TA98); with metabolic activation, 2-aminoanthracene served as the positive control for all strains.	Negative.	Zeiger et al. 1992
Mixed Cresols	TA98, TA100, and TA1537	Ames assay with and without S9 activation from Aroclor-induced rats; tested from 0.005 to 50 $\mu\text{l}/\text{plate}$ .	Negative.	Litton Bionetics, Inc. 1980a
m-Cresol	TA98, TA100, TA1535, and TA1537	Ames test with and without S9 from Aroclor-induced rats; tested at 0.03, 0.3, 3, and 30 $\mu\text{M}/\text{plate}$ .	Negative.	Florin et al. 1980
m-Cresol	Not stated	With metabolic activation from Aroclor 1254-induced rat-liver homogenate (S9); tested at 2 mg per plate.	Presumably nonmutagenic but solubility did not allow the testing of the compounds in amounts that result in lethality.	Nestmann et al. 1980

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**TABLE 9**  
Genotoxicity of Cresols and Isopropyl Cresols in *Salmonella typhimurium*

Ingredient	Strains	Protocol and dose	Results	Reference
<i>m</i> -Cresol	TA98	Liquid preincubation assay; tested at 5 doses ranging from ~1 to 1000 $\mu$ g/plate. Positive controls were used.	Nontoxic, but promotive to mutagenicity.	Abe and Urano 1993
<i>m</i> -Cresol	TA98, TA100, TA1535, TA1537, and TA1538	With and without S9 activation; tested at 0, 5, 50, 500, and 5000 $\mu$ g/plate.	Negative.	Pool and Lin 1982
<i>m</i> -Cresol	TA98, TA100, TA1535, and TA1537	With and without activation from Aroclor 1254-induced male Syrian hamster liver S9 or Sprague-Dawley rat liver S9; tested at 5 doses at half-log intervals up to 10,000 $\mu$ g/plate.	Negative.	Case Western Reserve 1981
<i>m</i> -Cresol	TA98, TA100, TA1535, and TA1537	A modified Ames test with and without rat and/or hamster S9. Positive controls were used. <i>m</i> -Cresol tested from 3.3 to 333 $\mu$ g/plate.	Negative.	Haworth et al. 1983
<i>m</i> -Cresol	TA98, TA100, TA1535, TA1537, and TA1538	Ames test with and without S9 with 2 mg <i>m</i> -Cresol.	Negative.	Douglas et al. 1980
<i>o</i> -Cresol	TA98 and TA100	Ames test with and without metabolic activation from liver supernatant (SP) prepared from 1254 Aroclor induced CD-1 rats (150–220 g).	Negative in strain TA100 with and without exogenous metabolic activation; positive in strain TA98 with and without exogenous metabolic activation.	Claxton 1985
<i>o</i> -Cresol	TA98, TA100, TA1535, and TA1537	Ames test with and without S9 from Aroclor-induced rats; tested at 0.03, 0.3, 3, and 30 $\mu$ M/plate.	Negative.	Florin et al. 1980
<i>o</i> -Cresol	TA98 and TA100	Ames test with and without S9 activation (from male rats treated with Aroclor 1254) using enzymatic oxidation at pH 4 and 7; tested at 0, 5.4, and 54 $\mu$ g.	Negative.	Massey et al. 1994
<i>o</i> -Cresol	TA98, TA100, TA1535, TA1537, and TA1538	Metabolic activation from Aroclor 1254-induced rat liver homogenate (S9); tested at 1 mg per plate.	Presumably nonmutagenic but solubility did not allow the testing of the compounds in amounts that result in lethality.	Nestmann et al. 1980
<i>o</i> -Cresol	TA98, TA100, TA1535, TA1537, and TA1538	With and without S9 activation; tested at 0, 5, 50, 500, and 5000 $\mu$ g/plate.	Negative.	Pool and Lin 1982
<i>o</i> -Cresol	TA98, TA100, TA1535, TA1537, and TA1538	Ames assay with and without S9 activation from Aroclor-induced rats; tested from 0.01 to 50 $\mu$ l/plate.	Negative.	Litton Bionetics, Inc. 1981

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## COSMETIC INGREDIENT REVIEW

**TABLE 9**  
Genotoxicity of Cresols and Isopropyl Cresols in *Salmonella typhimurium*

Ingredient	Strains	Protocol and dose	Results	Reference
<i>o</i> -Cresol	TA98, TA100, TA1535, and TA1537	With and without activation from Aroclor 1254-induced male Syrian hamster liver S9 or Sprague-Dawley rat liver S9; tested at 5 doses at half-log intervals up to 10,000 $\mu$ g/plate.	Negative.	Case Western Reserve 1981
60% <i>o</i> -Cresol	TA98, TA100, TA1535, TA1537, and TA1538	Ames test with and without S9 activation at doses of 5 to 500 $\mu$ g/plate; positive and negative controls gave appropriate responses.	Negative.	Tenneco Chemicals Inc. 1980b
<i>o</i> -Cresol	TA98, TA100, TA1535, TA1537, and TA1538	Ames test with and without S9. Dose limited to 2.5 $\mu$ l by solubility constraints.	Negative.	Douglas et al. 1980
<i>o</i> -Cresol	TA98, TA100, TA1535, and TA1537	A modified Ames test with and without rat and/or hamster S9. Positive controls were used. <i>o</i> -Cresol tested from 1 to 100 $\mu$ g/plate.	Negative.	Haworth et al. 1983
<i>p</i> -Cresol	TA98	Ames test with and without S9 from Aroclor-induced rats; tested at 0.03, 0.3, 3, and 30 $\mu$ M/plate.	Negative at lower doses and toxic at 30 $\mu$ M/plate.	Florin et al. 1980
<i>p</i> -Cresol	TA98 and TA100	Ames test with and without S9 activation (from male rats treated with Aroclor 1254) using enzymatic oxidation at pH 4 and 7; tested at 0, 5.4, and 54 $\mu$ g.	Negative.	Massey et al. 1994
<i>p</i> -Cresol	Not stated	With metabolic activation from Aroclor 1254-induced rat liver homogenate (S9); tested at 2.6 mg per plate (2.5 $\mu$ l).	Presumably nonmutagenic but solubility did not allow the testing of the compounds in amounts that result in lethality.	Nestmann et al. 1980
<i>p</i> -Cresol	TA98, TA100, TA1535, TA1537, and TA1538	Ames test with and without metabolic activation; tested at 0.03, 0.3, 3, and 30 $\mu$ M/plate.	Negative.	Battelle's Columbus Laboratories 1978
<i>p</i> -Cresol	TA98, TA100, TA1535, TA1537, and TA1538	With and without S9 activation; tested at 0, 5, 50, 500, and 5000 $\mu$ g/plate.	Negative.	Pool and Lin 1982
<i>p</i> -Cresol	TA98, TA100, TA1535, and TA1537	With and without activation from Aroclor 1254-induced male Syrian hamster liver S9 or Sprague-Dawley rat liver S9; tested at 5 doses at half-log intervals up to 10,000 $\mu$ g/plate.	Negative.	Case Western Reserve 1981
<i>p</i> -Cresol	TA98, TA100, TA1535, and TA1537	A modified Ames test with and without rat and/or hamster S9. Positive controls were used. <i>p</i> -Cresol tested from 33.3 to 333 $\mu$ g/plate.	Negative.	Haworth et al. 1983

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**TABLE 9**  
Genotoxicity of Cresols and Isopropyl Cresols in *Salmonella typhimurium*

Ingredient	Strains	Protocol and dose	Results	Reference
<i>p</i> -Cresol	TA98, TA100, TA1535, TA1537, and TA1538	Ames test with and without S9. Dose limited to 1 mg by solubility constraints.	Negative.	Douglas et al. 1980
Thymol	TA98 and TA100	Ames test with and without S9 from Aroclor-induced rats; 0.1 M Thymol tested at 6.25, 12.5, 25.0 $\mu$ l/plate.	Slightly positive (regardless of metabolic activation).	Stammati et al. 1999
Thymol	TA98, TA100, TA1535, and TA1537	Ames test with and without phenobarbitone induced rat liver S9 preparation; tested at 0.03, 0.3, 3, and 30 $\mu$ M/plate.	Negative.	Florin et al. 1980
Thymol	TA97, TA98, and TA100	Ames test with and without S9 metabolic activation; Thymol tested at a concentration of 1 mg/ml; positive and negative control gave appropriate responses.	Negative (regardless of metabolic activation).	Azizan and Blevins 1995
<i>o</i> -Cymen-5-ol	TA98, TA100, TA1535, TA1537, and TA1538	1 to 800 $\mu$ g/plate of <i>o</i> -Cymen-5-ol using the preincubation method with and without metabolic activation. Positive and negative controls were used.	Negative.	Osaka City Institute of Public Health and Environmental Sciences 1981
Carvacrol	TA98 and TA100	Ames test with and without S9 from Aroclor-induced rats; 0.1 M Carvacrol tested at 6.25, 12.5, 25.0 $\mu$ l/plate.	Slightly positive (regardless of metabolic activation).	Stammati et al. (1999)

4 weeks with refeeding twice a week. The assay was terminated by fixing the cell monolayers with methanol and staining with Giemsa. The stained cells were evaluated for the number of transformed foci.

Mixed Cresols induced an absolute and dose dependent increase in transformed foci over the applied concentration range of 0.01 to 8.0 nl/ml. However, the data were only statistically significant at the highest concentration (8.0 nl/ml). The applied concentration range corresponded to 62% to 50% survival in the cytotoxicity assay. Mixed Cresols was considered active in the BALB/c 3T3-rat hepatocyte-mediated transformation assay (Litton Bionetics, Inc. 1980a).

Litton Bionetics, Inc. (1980a) investigated the ability of Mixed Cresols to induce sister-chromatid exchanges (SCEs) in CHO cells with and without metabolic activation. The cultured cells were treated with Mixed Cresols in DMSO at concentrations between 0.5 to 100 nl/ml and grown with 5-bromodeoxyuridine (BrdU) for about a day. The chromosome preparations were then stained for SCEs. Positive and negative controls were used.

Mixed Cresols was able to induce significant increases in SCEs with and without metabolic activation, but was much less effective inducing SCEs in the presence of the S9. There were significant increases in SCEs at concentrations greater than 125 nl/ml Mixed Cresols without S9 cells. However, cells with S9 required concentrations greater than 200 nl/ml to result in significant increases in SCEs. This may have been due to the direct inactivation of the active components of Mixed Cresols by binding to proteins in the liver microsome fraction, or to partial detoxification by the microsomal enzymes (Litton Bionetics, Inc. 1980a).

Litton Bionetics, Inc. (1980b) examined the ability of Mixed Cresols to induce unscheduled DNA synthesis (UDS) in primary rat hepatocytes. Positive and negative (DMSO) controls were used. Mixed Cresols induced detectable UDS in primary rat hepatocytes at applied concentrations of 0.5 and 1.0 nl/ml. The number of nuclei with 6 or more grains and 20 or more grains were significantly increased. A smaller response was observed at 5.0 nl/ml and UDS was not detectable for treatments from 10 to 50 nl/ml. Treatment with 100 nl/ml Mixed Cresols was toxic.

Mixed Cresols was considered weakly active in the primary rat hepatocyte UDS assay.

Cheng and Kligerman (1984) evaluated the genotoxic potential of *m*-Cresol, *o*-Cresol, and *p*-Cresol using mammalian cells both in vitro and in vivo using the SCE assay. Induction of SCEs from Cresol exposure was tested in cultured human fibroblasts and mouse bone marrow, alveolar macrophages, and regenerating liver cells.

SCE frequency and average generation time (AGT) in cultured human fibroblasts were measured. Tested concentrations for *m*-Cresol, *o*-Cresol, or *p*-Cresol were 0 (ethanol control), 0.8, 4, 8, 10, and 30 mM. Mitomycin C dissolved in phosphate-buffered saline was used as a positive control. A 50- $\mu$ l volume of *m*-Cresol, *o*-Cresol, or *p*-Cresol was dissolved in 95% ethanol for concentrations under 10 mM, at greater concentrations the Cresol isomer was dissolved in minimum essential medium. Cells were allowed to attach for 4 h (at 37°C in a 5% CO<sub>2</sub>). Colcemid was added to each culture to stop cells at metaphase.

At concentrations of 10 mM, cytotoxicity was manifested by cells detaching from the plate when exposed to *p*-Cresol, the lack of cell division when exposed to *m*-Cresol, and the presence of only first division metaphases when exposed to *o*-Cresol. The 10 mM concentration of *o*-Cresol induced a small increase in SCEs in fibroblasts, but the authors postulated this had little biological relevance when the size of the effect is considered in relation to the *o*-Cresol concentration in the medium. SCEs were not induced at any concentration of *m*-Cresol, *o*-Cresol, or *p*-Cresol.

These authors also used male DBA/2NCrIb mice (3.5 to 4 months old weighing  $25.4 \pm 2.8$  g) for analysis of the effects of *m*-Cresol, *o*-Cresol, and *p*-Cresol on SCEs in bone marrow cells, alveolar macrophages, and regenerating liver cells. Water and a NIH-07 diet was available ad libitum until the beginning of the experiment. Approximately half of the animals were partially hepatectomized 5 days prior to exposure to induce liver cell regeneration. Mice received a single ip injection ( $\leq 0.35$  ml volume) of Cresol isomer dissolved in sunflower oil. The *m*-Cresol, *o*-Cresol, and *p*-Cresol doses were respectively 200 (six mice tested), 200 (five mice tested), and 75 (six mice tested) mg/kg. Nine positive control animals received only sunflower oil. Two positive control animals were injected with 5 mg cyclophosphamide/kg by a single ip injection. Thirty minutes later, 29 mg BrdU tablets were implanted subcutaneously for DNA labeling over 17 h. Animals were then administered a single ip injection of 3.3 mg colchicine/kg and were killed 4 h later. Twenty metaphases were analyzed for each cell type from each animal.

The data showed a complete absence of genotoxic effects from *m*-Cresol, *o*-Cresol, and *p*-Cresol in vivo in bone marrow, alveolar macrophages, and regenerating liver cells. The authors postulated the absence of genotoxic effects from Cresols may be due to their rapid conjugation with glucuronide or organic sulfates (Cheng and Kligerman 1984).

Jansson et al. (1986) investigated induction of SCEs in human lymphocytes using *m*-Cresol, *o*-Cresol, and *p*-Cresol iso-

lated from cigarette smoke condensate. Distillation of the condensate was performed at two different temperatures (20°C and 80°C) and yielded three fractions: volatiles (10%), semivolatiles (20%), and nonvolatiles (60%). The semivolatile, weakly acidic fraction was separated and contained 11 subfractions (F1 to F11) and 37 chemicals. The main components of fraction F8 were phenol, *m*-Cresol, and *p*-Cresol; the main components of F9 were *o*-Cresol and other unnamed multialkylated phenols.

Fractions F8 (concentration range from 0 to 80 mM) and F9 (concentration range from 0 to 70 mM) induced SCEs in a dose-dependent way. Fraction F8 produced the lowest activity of all fractions tested. However, when *m*-Cresol, *o*-Cresol, and *p*-Cresol were separated and tested at concentrations of 0.0 to 0.5, 0 to 1.0, and 0 to 0.5 mM, respectively, they did not induce SCEs in human lymphocytes (Jansson et al. 1986).

*m*-Cresol. Levan and Tjio (1948) tested *m*-Cresol in *Allium* for chromosome aberrations at a concentration up to 1.0 mol/L; the test was negative.

Sharma and Ghosh (1965) tested *m*-Cresol in *Allium* for chromosome aberrations at concentrations of 0.025%, 0.02%, and 0.015% (in distilled water); the test was positive. Roots became flaccid after 24 h treatment with the two greater concentrations tested and they showed c-tumor, associated with some polyploid cells, after revival. Ball and star metaphases, good clearing of the chromosomes, contraction and diplochromatid appearance of the chromosomes were observed at various concentrations. Reviving root cells showed multipolar arrangement, multinucleate cells, clumping, stickiness, and sticky bridges. Up to 19.23% of cells contained chromosome fragments (0.025% group after 5-h treatment period).

The Chemical Industry Institute of Toxicology (CIIT 1983) determined the genotoxic potential of *m*-Cresol using an SCE assay in vitro and in vivo. In vitro, *m*-Cresol did not cause any statistically significant increase in SCEs in human diploid fibroblasts at the concentrations tested (0 to 0.01 M). *m*-Cresol did not induce significant cell-cycle inhibition. In vivo, to determine whether *m*-Cresol was capable of inducing SCEs, male DBA mice (intact or subjected to a 2/3 partial hepatectomy 5 days earlier) were injected intraperitoneally with 200 mg/kg (approximately half the LD<sub>50</sub>) *m*-Cresol in sunflower oil. A 29-mg pellet of bromodeoxyuridine was implanted subcutaneously 1 h after injection. Colchicine was administered intraperitoneally 17 h later, and the animals were killed at 22 h post injection. Bone marrow, alveolar macrophages, and regenerating liver cells were scored for SCE frequencies. No significant increases in SCE frequency over controls were found in any of the exposed groups in any of the organs examined.

Hazelton Laboratories, Inc. (1988b) evaluated the ability of *m*-Cresol to induce chromosome aberrations in CHO cells with and without metabolic activation. The chromosomal aberrations assay without metabolic activation was tested using *m*-Cresol at concentrations from 198 to 495  $\mu$ g/ml. *m*-Cresol was tested at concentrations from 250 to 1100  $\mu$ g/ml with metabolic activation. Mitomycin C was used for the nonactivation studies

and cyclophosphamide was used for the activation studies as the positive controls. McCoy's 5a culture medium was used as the negative control.

In the *m*-Cresol assay without metabolic activation, there was an unhealthy monolayer, floating dead cells, and few visible mitotic cells at 495  $\mu\text{g/ml}$ . An unhealthy monolayer was also observed at 297 and 396  $\mu\text{g/ml}$ . However, there was no significant increase in chromosomal aberrations at the concentrations tested and *m*-Cresol was considered negative under nonactivation conditions. In the *m*-Cresol assay with metabolic activation, there was only a significant increase in chromosomal aberrations at 999  $\mu\text{g/ml}$ , which was the maximum test concentration in the first trial.

The assay was repeated with concentrations ranging from 699 to 1100  $\mu\text{g/ml}$ . Complete mitotic suppression was observed at concentrations greater than 898  $\mu\text{g/ml}$ . Due to the shift in toxicity in the second trial, results were analyzed only at 699 and 799  $\mu\text{g/ml}$ . A weak increase in chromosomal aberrations was observed at 799  $\mu\text{g/ml}$ ; because the significant increase in chromosomal aberrations was inconsistent between the two trials and no dose response was established in either trial, *m*-Cresol was considered negative for inducing chromosomal aberrations under conditions of metabolic activation (Hazelton Laboratories 1988b).

Hazelton Laboratories (1988c) examined the ability of *m*-Cresol to induce UDS in primary rat hepatocytes. Positive (1-acetyl aminofluorene) and negative (DMSO) controls were used. Hepatocytes were exposed to *m*-Cresol for 18.5 h at concentrations ranging from 0.10 to 502  $\mu\text{g/ml}$  in the presence of thymidine. The treatments from 25.1 to 502  $\mu\text{g/ml}$  were excessively toxic and did not allow for autoradiographic analysis of nuclear labeling. At 10.0  $\mu\text{g/ml}$ , 72.4% survival was obtained and survival increased up to 92.2% at 5.02  $\mu\text{g/ml}$  of *m*-Cresol. No toxicity was observed at 2.51  $\mu\text{g/ml}$ . Analysis of cultures exposed from 0.251 to 10.0  $\mu\text{g/ml}$  showed no evidence of UDS. *m*-Cresol was considered inactive in the primary rat hepatocyte UDS assay.

This laboratory (Hazelton Laboratories 1988d) also tested *m*-Cresol in the L5178Y mouse lymphoma cell assay. L5178Y/TK<sup>+/-</sup> cells were treated with 13 to 520  $\mu\text{g/ml}$  of *m*-Cresol without metabolic activation for 4 h. Negative control cells were treated with dimethylsulfoxide alone. Following cell treatment and rinsing, cultures were placed in growth medium for 2 days to allow for growth and expression of the induced TK<sup>-/-</sup> phenotype. Cell counts were done daily and appropriate dilutions were made to maximize growth. Following a 12-day incubation period, the colonies were sized and counted. L5178Y/TK<sup>+/-</sup> cells were treated with 52 to 520  $\mu\text{g/ml}$  of *m*-Cresol with metabolic activation. For metabolic activation, the only difference in the protocol was the addition of the S9 fraction of rat liver homogenate and necessary cofactors during the 4-h treatment period. The components of the activation system in the cell suspension assays were NADP (sodium salt), isocitric acid, and S9. Ethylmethane sulfonate was the positive control used

for the nonactivation studies and 3-methylcholanthrene was the positive control used for the metabolic activation studies.

*m*-Cresol was not mutagenic in the mouse lymphoma forward mutation assay with and without metabolic activation. At the highest concentration tested (520  $\mu\text{g/ml}$ ), *m*-Cresol was lethal with and without metabolic activation. Therefore, *m*-Cresol was analyzed with and without metabolic activation at the following concentrations: 52, 104, 156, 260, 312, and 416  $\mu\text{g/ml}$ . Without metabolic activation, *m*-Cresol induced toxicities from 18.5% to 71.0% at concentrations from 52 to 416  $\mu\text{g/ml}$ . With metabolic activation, *m*-Cresol induced toxicities from 6.7% to 55.4% at concentrations from 52 to 416  $\mu\text{g/ml}$ . However, *m*-Cresol was not able to increase mutation frequencies by more than twofold over the average solvent control mutation frequency (Hazelton Laboratories 1988d).

Hazelton Laboratories (1988e) tested *m*-Cresol in the in vitro transformation of 3T3 cells with metabolic activation. A series of 25-cm<sup>2</sup> flasks were each seeded with  $5 \times 10^4$  3T3 cells and incubated for 24 h. Afterward, rat liver cells were seeded into each culture and after a 3-h incubation period, flasks were treated with one of the five preselected concentrations of *m*-Cresol (6, 12, 24, 48, and 72 nl/ml). Positive (dimethylnitrosamine with and without TPA) and negative (Eagle's minimum essential medium) controls were also prepared. The flasks were incubated for 2 days and the cells were then rinsed. Incubation then continued for 28 days with refeeding twice a week. The stained cells were examined and the number of transformed foci were determined.

*m*-Cresol in the range of concentrations tested from 6 to 72 nl/ml was found to produce cytotoxicity from 7.2% to 100.3% over the same concentrations. However, *m*-Cresol did not produce significant increases in the number of transformed foci considered to indicate a positive response. Therefore, *m*-Cresol was negative at the concentrations tested to induce morphological cell transformation of BALB/c 3T3 cells assay with metabolic activation. The controls gave the expected results (Hazelton Laboratories 1988e).

In a similar in vitro experiment, Hazelton Laboratories (1988f) tested *m*-Cresol transformation of 3T3 cells without metabolic activation. A series of 25-cm<sup>2</sup> flasks were each seeded with  $1 \times 10^4$  to  $3 \times 10^4$  3T3 cells and incubated for 24 h. Afterward, rat liver cells were seeded into each culture and after a 3-h incubation period, flasks were treated with one of the five preselected concentrations of *m*-Cresol (0.57, 3.4, 8.5, 17, and 34 nl/ml). Positive (3-methylcholanthrene) and negative (Eagle's minimum essential medium) controls were also prepared. The flasks were incubated for 2 days and then rinsed. Incubation then continued for about 4 weeks with refeeding twice a week. The stained cells were then examined and the number of transformed foci were determined. Three trials were performed due to shifts in toxicity and variable increases in foci.

*m*-Cresol was relatively toxic to 3T3 cells in the trials. In trial 1, *m*-Cresol in the range of concentrations tested from 0.57 to 34 nl/ml, produced cytotoxicity from 3.7% to 45.1%. This assay was inconclusive because it showed two concentrations (3.4

and 8.5 nl/ml) with increased foci that were not dose-related and occurred at nontoxic concentrations. Therefore, trial 2 was performed at an increased concentration range of 4.0 to 48.0 nl/ml; however, all the data were unacceptable due to an increase of background transformations. Trial 3 was performed using the concentration range of 4.0 to 48.0 nl/ml. There were no surviving colonies above the 12.0 nl/ml concentration and there was no evidence of transforming activity. *m*-Cresol produced negative results since there was no reproducible dose-related induction of transforming activity (Hazelton Laboratories 1988f).

Hazelton Laboratories (1989b) investigated the ability of *m*-Cresol to induce chromosomal aberrations in the bone marrow of 8- to 10-week-old ICR mice. *m*-Cresol in corn oil was administered to mice by gavage at 96, 320, and 960 mg/kg. Mice were killed at 6, 24, and 48 h after dosing for extraction of bone marrow. Positive (80 mg cyclophosphamide) and negative (corn oil only) controls were used at each time. Ten animals (5 male and 5 female) were used for each study group and weighed 23.9 to 38.2 g and 22.0 to 30.8 g for the males and females, respectively.

After 10 min, all mice exposed to 960 mg/kg *m*-Cresol had squinty eyes and a majority had mild tonic convulsions with rapid breathing. After 30 min the convulsions and rapid breathing stopped, but mice were lethargic, and had squinty eyes and scruffy coats. Three mice died in the 960 mg/kg dose group (at 7, 30, and 48 h). Mice dosed with 230 mg/kg of *m*-Cresol developed slightly scruffy coats within 22 h after dosing. All lower dose animals and controls appeared normal after dosing. A significant reduction in the mean mitotic index was induced in the positive control groups and chromosomal aberrations in mice bone marrow cells were not observed in negative controls. *m*-Cresol did not induce significant reductions in the mitotic index in any treatment group tested. *m*-Cresol did not induce any significant increase in the percentage of chromosomal aberrations in either sex, at any sacrifice time, or at any dose level in this assay as compared to controls. *m*-Cresol was considered negative for inducing chromosomal aberrations in bone marrow cells of male and female mice under the conditions of this study (Hazelton Laboratories 1989b).

Hamaguchi and Tsutsui (2000) examined the ability of *m*-Cresol to induce UDS using cultured Syrian hamster embryo (SHE) cells. *m*-Cresol was tested at concentrations of 1, 3, and 10  $\mu$ M in the presence and absence of exogenous metabolic activation. At levels that reduced survival by 10% or less, a dose-dependent increase in UDS levels was seen with metabolic activation, but not without activation.

*o*-Cresol. *o*-Cresol was tested for chromosome aberrations in *Allium* at a concentration up to 1.0 mol/L; the test was weakly positive (Levan and Tjio 1948).

Sharma and Ghosh (1965) tested *o*-Cresol in *Allium* for chromosome aberrations at concentrations of 0.025% and 0.01% (in distilled water); the test was negative. Roots became flaccid after 24-h treatments with 0.025% and 0.15% solutions. Chromosomes were clumped at greater concentrations and star shaped and clumped metaphases were common. At low doses, abnor-

malities such as clumping, stickiness, and sticky bridges were observed, but at high doses the roots died. No chromosome fragments could be detected.

The EG&G Mason Research Institute (1980) evaluated R-1044 (60% *o*-Cresol) using the C3H/10T<sub>1/2</sub> cell transformation system at four concentrations ranging from 0.0195 to 0.156  $\mu$ l/ml. The results indicated that R-1044 did not cause morphological transformation of cells in the C3H/10T<sub>1/2</sub> cell transformation assay. The solvent control did not show any indication of transformed foci while the positive control (DMBA) showed development of type II and type III transformed foci.

Tenneco Chemicals, Inc. (1980a) evaluated R-1044 (60% *o*-Cresol and 40% guaiacol and alkylphenol by weight) in the *Escherichia coli* DNA repair-suspension assay. R-1044 was dissolved in DMSO at 50 mg/ml, and lower concentrations (0.5, 1, 5, and 10 mg/ml) were prepared by serial dilution with DMSO. One-tenth-milliliter aliquots of these solutions were used at 50, 100, 500, 1000, and 5000  $\mu$ g/ml of bacterial suspension. Positive controls were 2-aminofluorene with metabolic activation and *N*-methyl-*N*-nitrosoguanidine without metabolic activation.

The survival index at all concentrations of R-1044, with and without metabolic activation, was greater than 0.80. The positive control with and without metabolic activation had a survival index of 0.69 and 0.54, respectively. R-1044 did not cause a significant preferential killing of the repair-deficient strain in the *E. coli* DNA repair-suspension assay (Tenneco Chemicals Inc. 1980a).

Litton Bionetics, Inc. (1981) studied the ability of *o*-Cresol to induce SCEs in CHO cells with and without metabolic activation. In tests without metabolic activation, the cultured cells were treated with *o*-Cresol in DMSO at concentrations between 12.5 to 100 nl/ml and grown with BrdU for about a day and then rinsed. In tests with metabolic activation, the cultured cells were treated with *o*-Cresol in DMSO at concentrations between 12.5 to 800 nl/ml and grown with BrdU for about 2 h and then rinsed. The chromosome preparations were then stained for detection of SCEs. Positive and negative controls were used.

*o*-Cresol induced significant increases in SCEs with and without metabolic activation. *o*-Cresol was considered positive in the SCE test but was much less effective inducing SCEs in the presence of the S9. There was a clear and significant increase in SCEs at *o*-Cresol concentrations of 50 nl/ml or more without S9 cells. The SCE rate at 75 nl/ml was almost double that of solvent controls. However, cells with S9 required concentrations from 500 to 700 nl/ml to see significant increases in SCEs. No results were obtained at 800 nl/ml due to complete toxicity. The author noted that the results of the two tests should not be compared directly due to the wide disparity in treatment lengths with and without metabolic activation (Litton Bionetics, Inc. 1981).

Litton Bionetics, Inc. (1981) also tested *o*-Cresol in the L5178Y mouse lymphoma cell assay. L5178Y/TK<sup>+/-</sup> cells were treated with 15.6 to 250 nl/ml of *o*-Cresol without metabolic activation for 4 h. Negative-control cells were treated with

dimethylsulfoxide alone. Following cell treatment and rinsing, cultures were placed in growth medium for 2 to 3 days to allow for growth and expression of the induced TK<sup>-/-</sup> phenotype. Cell counts were done daily and appropriate dilutions were made to maximize growth. Following a 10-day incubation period, the colonies were sized and counted. L5178Y/TK<sup>+/-</sup> cells were treated with 3.91 to 62.5 nl/ml of *o*-Cresol with metabolic activation. For metabolic activation, the only difference in the protocol was the addition of the S9 fraction of rat liver homogenate and necessary cofactors during the 4-h treatment period. The components of the activation system in the cell suspension assays were NADP (sodium salt), isocitric acid, and S9. Ethylmethane sulfonate was the positive control used for the nonactivation studies and dimethylnitrosamine was the positive control used for the metabolic activation studies.

*o*-Cresol did not induce significant increases in the mutant frequency at the TK locus in the L5178Y mouse lymphoma cells. Concentrations of *o*-Cresol ranged from 15.6 to 250 nl/ml without metabolic activation and 3.91 to 62.5 nl/ml in the presence of rat liver S9 microsomal activation. With and without metabolic activation, highly toxic amounts were reached without any significant mutagenic activity. *o*-Cresol was considered inactive in the mouse lymphoma forward mutation assay (Litton Bionetics, Inc. 1981).

This same laboratory tested *o*-Cresol in the in vitro transformation of BALB/c 3T3 cells with activation by primary rat hepatocytes. A series of flasks were seeded with primary rat hepatocytes and incubated for 24 h. Afterward, 3T3 cells were seeded into each culture and after a 24-h cocultivation period, at least 20 flasks were treated with one of the five preselected concentrations of *o*-Cresol (0.264, 2.64, 10.6, 42.3, and 169.0 µg/ml). At least 20 positive (3-methylanthracene) and 20 negative (DMSO) controls were also prepared for each assay. The flasks were incubated for 2 days and the cells were then rinsed. Incubation then continued for 4 weeks, with refeeding twice a week, after which the cells were stained. The stained cells were then examined and the number of transformed foci were determined.

*o*-Cresol did not induce significant increases in the number of transformed foci for an applied concentration range of 0.264 to 169 µg/ml. The concentration range corresponded to approximately 20% to 90% survival in the cytotoxicity test. *o*-Cresol was considered to be inactive in the BALB/c 3T3 in vitro transformation assay.

The ability of *o*-Cresol to induce UDS in primary rat hepatocytes also was examined. Positive and negative (DMSO) controls were used. *o*-Cresol did not induce detectable UDS in primary rat hepatocytes for applied concentrations from 0.5 to 50 nl/ml. These treatments resulted in a cell survival range from 100.6% to 45.6%. Treatment with 100 nl/ml *o*-Cresol was excessively toxic and insufficient cells were available for analysis of nuclear labeling. *o*-Cresol was considered inactive in the primary rat hepatocyte UDS assay (Litton Bionetics, Inc. 1981).

The genotoxic potential of *o*-Cresol was determined using SCE assay in vitro and in vivo (CIIT 1983). In vitro, *o*-Cresol

caused a statistically significant increase in SCEs in human diploid fibroblasts at the highest concentration tested (0.01 M). The biological significance of this is questionable because of the high concentration, the lack of dose-response, and the small increase in SCEs observed. *o*-Cresol did not induce significant cell-cycle inhibition. In vivo, to determine if *o*-Cresol was capable of inducing SCEs, male DBA mice (intact or subjected to a 2/3 partial hepatectomy 5 days earlier) were injected intraperitoneally with 200 mg/kg (approximately half the LD<sub>50</sub>) *o*-Cresol in sunflower oil. A 29-mg pellet of BrdU was implanted subcutaneously 1 h after injection. Colchicine was administered intraperitoneally 17 h later, and the animals were killed at 22 h post injection. Bone marrow, alveolar macrophages, and regenerating liver cells were scored for SCE frequencies. No significant increases in SCE frequency over controls was found in any of the organs examined from the exposed groups.

Hazleton Laboratories (1988a) tested *o*-Cresol in the in vitro transformation of BALB/c 3T3 cells assay with metabolic activation. A series of 25-cm<sup>2</sup> flasks were seeded with 5 × 10<sup>4</sup> BALB/c 3T3 cells/flask and incubated for 24 h. Afterward, rat liver cells were each seeded into each culture and after a 3-h incubation period, flasks were treated with each of the five preselected concentrations of *o*-Cresol (7.5, 15, 22.5, 30, and 45 nl/ml). Positive (dimethylnitrosamine with and without TPA) and negative (Eagle's minimum essential medium) controls were also prepared. The flasks were incubated for 2 days and then rinsed. Incubation then continued for 36 days with refeeding twice a week. The stained cells were then examined and the number of transformed foci were determined.

*o*-Cresol, in the range of concentrations tested from 7.5 to 45 nl/ml, was found to produce cytotoxicity from 7.2% to 87.8% over the same concentrations. However, the only significant increase in foci was observed at 15 nl/ml and was not concentration-related; therefore, *o*-Cresol was not considered to cause cell transformation at the concentrations tested. The controls gave the expected results (Hazleton Laboratories 1988a).

Hazleton Laboratories (1988b) evaluated the ability of *o*-Cresol to induce chromosome aberrations in CHO cells with and without metabolic activation. The chromosomal aberration assay without metabolic activation was completed using *o*-Cresol at concentrations from 100 to 300 µg/ml. *o*-Cresol was tested at concentrations from 50 to 1000 µg/ml with metabolic activation and cells were fixed at 10 or 19.9 h after treatment. Mitomycin C was used for the nonactivation studies and cyclophosphamide was used for the activation studies as the positive controls. McCoy's 5a culture medium was used as the negative control.

In the *o*-Cresol assay without metabolic activation, there were unhealthy monolayers at concentrations of 100 to 300 µg/ml. There was extreme toxicity at the 300 µg/ml concentration. There were significant increases in chromosomal aberrations at 150, 200, and 250 µg/ml and *o*-Cresol was evaluated as positive for inducing chromosome aberrations under nonactivation

conditions. In the *o*-Cresol assay with metabolic activation, there was complete toxicity at 1000  $\mu\text{g/ml}$  in the 19.9-h aberrations assay. An unhealthy monolayer was observed at concentrations of 750  $\mu\text{g/ml}$ , but there was no toxicity at 500  $\mu\text{g/ml}$ .

There were significant increases in chromosomal aberrations at 500 and 750  $\mu\text{g/ml}$  in the 19.9-h assay. Complex chromatid type aberrations such as quadriradials and triradials were observed in the treated cells. In the 10-h assay, there was an unhealthy monolayer with a reduction in the number of mitotic cells at 500  $\mu\text{g/ml}$  but there was no toxicity at 50, 125, 250, and 375  $\mu\text{g/ml}$ . There were only significant increases in chromosomal aberrations at 500  $\mu\text{g/ml}$  in the 10-h assay. *o*-Cresol was positive for inducing chromosomal aberrations under conditions of metabolic activation (Hazelton Laboratories 1988b).

*o*-Cresol was retested in the in vitro transformation of BALB/c 3T3 cells assay with metabolic activation by Hazelton Laboratories (1989a). The procedures were previously described. *o*-Cresol in the range of concentrations tested, from 7.5 to 45  $\text{nl/ml}$ , was found to produce cytotoxicity from 7.2% to 87.8%. *o*-Cresol, at the highest concentration tested (45  $\text{nl/ml}$ ), induced a significant increase in the number of transformed foci. A small increase was also seen at 22.5  $\text{nl/ml}$  (95% cell survival) but not at 30  $\text{nl/ml}$  (46% cell survival).

In the second trial, *o*-Cresol caused cell transformation. There was a clear increase at the highest concentration tested of *o*-Cresol (45  $\text{nl/ml}$ ). The average number of transformed foci per culture increased from 0.11 for the solvent control to 0.94 for treated cultures. However, the data were considered insufficient to assess if the transfer of *o*-Cresol metabolites by the rat liver cells to the target BALB/c 3T3 cells was due to random variation or a sporadic activation. *o*-Cresol transforming activity in the transformation of BALB/c 3T3 cells assay with metabolic activation was considered inconclusive due to the results in the 1st and 2nd trials.

Hazelton Laboratories (1989c) tested the ability of *o*-Cresol to cause fetal death in untreated female mice following mating to males acutely treated with *o*-Cresol. Eight-week-old ICR mice were used for the study and food and water were available ad libitum. Male mice (25/group) were dosed by gavage with 75, 250, or 750  $\text{mg/kg}$  *o*-Cresol in corn oil. Males were then mated to females for 6 consecutive weeks to assess dominant lethal effects at all stages of male germ cell development. At about 19 h after dosing, males were mated to the first mating group of two females for 5 days. The males were then rested for 2 days before being introduced to a new mating group. The females were killed 14 days after the midweek of mating and were examined for number of live embryos and the number of dead embryos in early and late gestation. Triethylenemelamine (TEM) was used as a positive control.

Eight male mice died in the high dose group within 5 days of dosing and other surviving male mice were languid with squinting eyes, but all surviving mice appeared normal by day 6. Animals dosed with 250  $\text{mg/kg}$  *o*-Cresol became languid 5 min after dosing and recovered within 1 h. All other dose groups

appeared normal and healthy. All female mice appeared healthy throughout the study.

No significant reductions in body weights were observed in any of the males of any dose group. There were no significant effects of *o*-Cresol at any dose level on the number of implantations, the number of dead implantations, the proportion of females with either one or more or two or more dead implantations, or on the frequency of dead embryos relative to the total number of embryos in each female. However, a significant reduction in fertility was detected in the first week of mating in the high dose group but this was expected because males were still suffering from symptoms such as lethargy, squinted eyes, rough haircoat, and distended abdomens. The positive control, TEM, induced a large and significant increase in the number of dead implantations, the proportion of females with either one or more or two or more dead implantations, or on the frequency of dead embryos relative to the total number of embryos in each female through the first three weeks of the study. *o*-Cresol was considered negative for inducing dominant lethal mutations in the germ cells of male mice (Hazelton Laboratories 1989c).

Hazelton Laboratories (1989g) investigated the mutagenic activity of *o*-Cresol using the *Drosophila melanogaster* Sex-Linked Recessive Lethal Test to detect the occurrence of gene point mutations in germ cells. Preliminary toxicity tests resulted in an  $\text{LD}_{50}$  of 1000  $\mu\text{g/ml}$ , therefore the doses of 100, 500, and 1000  $\mu\text{g/ml}$  were selected for the Sex-Linked Recessive Lethal Test using *o*-Cresol. Some of the surviving males were tested for fertility by mating each to three Basc virgin females, *o*-Cresol had no effect on fertility. *o*-Cresol (dissolved in 5% aqueous sucrose) was administered orally to flies for up to 3 days. There were 6 to 10 vials each containing 25 flies per treatment group. The negative control was 5% sucrose solution and the positive control was 0.005 M ethyl methanesulfonate in 5% sucrose solution. Treated males were mated individually to sequential groups of three virgin Basc females and the brooding schedule consisted of 3, 2, 2 day sequence that samples mature and immature sperm.

At 100  $\mu\text{g/ml}$  there were 3 recessive lethals out of 4476 (0.06%) X-chromosomes tested, at 500  $\mu\text{g/ml}$  there was 1 recessive lethal out of 4620 (0.02%) X-chromosomes tested, and at 1000  $\mu\text{g/ml}$  there were 6 recessive lethals out of 5778 (0.10%) X-chromosomes tested. Using trend analysis, *o*-Cresol was not significantly mutagenic as compared to controls (Hazelton Laboratories 1989g).

*p*-Cresol. *p*-Cresol was tested for chromosomal aberrations in *Allium* at a dose of up to 1.0  $\text{mol/L}$ ; the test was weakly positive (Levan and Tjio 1948).

Sharma and Ghosh (1965) tested *p*-Cresol in *Allium* for chromosomal aberrations at concentrations of 0.025%, 0.01%, and 0.0075% (in distilled water); the test was negative. C-mitosis was obtained with high dosage, associated with lower stainability, diplochromatid appearance, and shortening of the chromosomes. In these cases division was rare and showed chromosome stickiness.

Battelle's Columbus Laboratories (1978) examined the ability of *p*-Cresol to induce UDS in WI-38 human embryonic lung fibroblasts by measuring the amount of tritiated thymidine incorporated into cells exposed to subtoxic amounts of *p*-Cresol. The amount of *p*-Cresol used was 0.1 to 0.82 mg. Only cells exposed to 0.82 mg of *p*-Cresol incorporated increased amounts of thymidine over control levels, which indicated repair of DNA damage caused by exposure. A second exposure of cells to *p*-Cresol confirmed the positive results from the first experiment.

These authors also used *p*-Cresol in the C3H/10T<sub>1/2</sub> cell transformation system at three levels ranging from 0.06 to 6.0  $\mu\text{g/ml}$ . *p*-Cresol did not produce significant increases in transformation above control cell levels at any of the concentrations tested. The cells exposed to the positive control, DMBA, responded in a dose-dependent manner (Battelle Columbus Laboratories 1978).

CIIT (1983) determined the genotoxic potential of *p*-Cresol using SCE assay in vitro and in vivo. In vitro, *p*-Cresol did not cause any statistically significant increase in SCEs in human diploid fibroblasts at the concentrations tested (0 to 0.01 M). *p*-Cresol did not induce significant cell-cycle inhibition. In vivo, to determine if *p*-Cresol was capable of inducing SCEs, male DBA mice (intact or subjected to a 2/3 partial hepatectomy 5 days earlier) were injected intraperitoneally with 75 mg/kg (approximately half the LD<sub>50</sub>) *p*-Cresol in sunflower oil. A 29-mg pellet of bromodeoxyuridine was implanted subcutaneously 1 h after injection. Colchicine was administered intraperitoneally 17 h later, and the animals were killed at 22 h post injection. Bone marrow, alveolar macrophages, and regenerating liver cells were scored for SCE frequencies. No significant increases in SCE frequency over controls was found in any of the exposed groups in any of the organs examined.

Daugherty and Franks (1986) investigated semiconservative DNA synthesis and DNA repair in human peripheral lymphocytes using *p*-Cresol as a possible inhibitor. Blood samples were collected from four male human donors, aged 18 to 46 years, and lymphocytes were isolated (1.0 mM hydroxyurea was used to inhibit semiconservative DNA synthesis during incubation). *p*-Cresol was tested from 5 to 25  $\mu\text{M}$ . The experiment was performed at least nine times. Twenty-five micromolar *p*-Cresol was found to inhibit semiconservative DNA synthesis and UV-induced DNA repair 25% and 21%, respectively (standard deviation was less than  $\pm 15\%$ ).

Hazelton Laboratories (1988f) tested *p*-Cresol in the in vitro transformation of BALB/c 3T3 cells assay without metabolic activation. A series of 25-cm<sup>2</sup> flasks were seeded with  $1 \times 10^4$  to  $3 \times 10^4$  BALB/c 3T3 cells/flask and incubated for 24 h. Afterward, rat liver cells were seeded into each culture and after a 3-h incubation period, the cells were treated with each of the five preselected concentrations of *p*-Cresol (0.81, 3.25, 5, 10, and 15 nl/ml). Positive (3-methylcholantrene) and negative (Eagle's minimum essential medium) controls were also prepared. The flasks were incubated for 2 days and the cells were then rinsed. Incubation continued for about 4 weeks with refeeding twice per

week. The stained cells were then examined and the number of transformed foci were determined.

*p*-Cresol, in the range of concentrations tested from 0.81 to 15 nl/ml, was found to produce cytotoxicity from 7.7% to 91.6%. *p*-Cresol produced a concentration-related increase in the number of foci per plate over the entire concentration range. *p*-Cresol was considered active in the BALB/c 3T3 cell transformation assay without metabolic activation (Hazelton Laboratories 1988f).

This same laboratory evaluated the ability of *p*-Cresol to induce chromosome aberrations in CHO cells with and without metabolic activation. The chromosomal aberrations assay without metabolic activation was tested using *p*-Cresol at concentrations from 100 to 301  $\mu\text{g/ml}$ . *p*-Cresol was tested at concentrations from 30 to 300  $\mu\text{g/ml}$  with metabolic activation in the 10-h assay and with 301 to 902  $\mu\text{g/ml}$  in the 20-h assay. Mitomycin C was used for the nonactivation studies and cyclophosphamide was used for the activation studies as the positive controls. McCoy's 5a culture medium was used as the negative control.

In the *p*-Cresol assay without metabolic activation, there were significant increases in chromosomal aberrations at all concentrations tested. No response was observed in the 10-h assay with metabolic activation. Significant increases were seen in the 20-h assay with metabolic activation at the 301 and 601  $\mu\text{g/ml}$  concentrations. The 902  $\mu\text{g/ml}$  concentration was toxic and could not be analyzed. *p*-Cresol was considered positive for inducing chromosomal aberrations in CHO cells under both activation and nonactivation conditions in this assay (Hazelton Laboratories 1988g).

Hazelton Laboratories (1989e) studied the ability of *p*-Cresol in a dominant lethal assay. Eight-week-old ICR mice were used for the study. Food and water were available ad libitum. Male mice (25/group) were administered a single dose by gavage with 100, 275, or 650 mg/kg *p*-Cresol in corn oil. Due to excessive toxicity in the high dose group, the high dose was decreased to 550 mg/kg using a new group of mice 2 weeks later. Males were then mated to females for 6 consecutive weeks to assess dominant lethal effects at all stages of male germ cell development. At about 19 h after dosing, males were mated to the first mating groups of two females for 5 days. The males were then rested for 2 days before being introduced to a new mating group. The females were killed 14 days after the midweek of mating and were examined for number of live embryos and the number of dead embryos in early and late gestation. Both positive (triethylenemelamine) and negative (corn oil) controls were used.

Seven male mice died in the 550 mg/kg group within 2 weeks of dosing and other remaining mice were languid with squinting eyes. The positive and negative controls and the 100 mg/kg *p*-Cresol dose group appeared normal and healthy. Mice in the 275 mg/kg group had scruffy coats. On the 2nd day of mating, low and medium dose groups and all controls appeared normal and healthy. All female mice appeared healthy throughout the study except four females that died in the gestation phase of the

study, two were from the low dose group and one was from the positive control group.

No significant reductions in body weights were observed in any of the males. There were no significant effects of *p*-Cresol at any dose level on the number of implantations, the number of dead implantations, the proportion of females with one or more dead implantations, or the frequency of dead embryos relative to the total number of embryos in each female. However, a significant increase in the percentage of pregnant females with more than one dead implant was observed in the 550 mg/kg group during the 4th mating week. This was not considered biologically significant since the comparative value of the 4th week for the vehicle control was low in comparison to the previous three weeks.

A significant reduction in fertility was detected in the first week of mating in the high dose group but this was expected because males were still suffering from symptoms such as lethargy, squinted eyes, rough haircoats, and distended abdomens. Fertility was also decreased in the 275 mg/kg dose groups and the positive control group in week 1 but no other effects were observed on fertility in any of the other test groups for the duration of the study. There was a decreased number of total embryos in the high dose group during week 3 of mating, which was due to a decreased number of males in the study. The positive control, TEM, induced a large and significant increase in the number of dead implantations, the proportion of females with one or more dead implantations, or the frequency of dead embryos relative to the total number of embryos in each female through the first three weeks of the study. Through the first 4 weeks, there was a significant decrease in the number of implantations in the positive control group. The negative control gave the expected results. *p*-Cresol was considered negative for inducing dominant lethal mutations in the germ cells of male mice (Hazelton Laboratories 1989e).

Hazelton Laboratories (1989h) investigated the mutagenic activity of *p*-Cresol using the *Drosophila melanogaster* Sex-Linked Recessive Lethal Test in order to detect the occurrence of gene point mutations in germ cells. Preliminary toxicity tests resulted in an LD<sub>50</sub> of 600 µg/ml, therefore the doses of 60, 300, and 600 µg/ml were selected for the Sex-Linked Recessive Lethal Test using *p*-Cresol.

Some of the surviving males were tested for fertility by mating each to three Basc virgin females, *p*-Cresol had no effect on fertility. *p*-Cresol (dissolved in 5% aqueous sucrose) was administered orally to flies for up to 3 days. There were 6 to 10 vials each containing 25 flies per treatment group. The negative control was 5% sucrose solution and the positive control was 0.005 M ethyl methanesulfonate in 5% sucrose solution. Treated males were mated individually to sequential groups of three virgin Basc females and the brooding schedule consisted of 3, 2, 2 day sequence that samples mature and immature sperm.

At 60 µg/ml there were three recessive lethals out of 5779 (0.05%) X-chromosomes tested, at 300 µg/ml there were three recessive lethals out of 5217 (0.06%) X-chromosomes tested,

and at 600 µg/ml there were 10 recessive lethals out of 5516 (0.18%) X-chromosomes tested. Using trend analysis, *p*-Cresol was not significantly mutagenic as compared to controls (Hazelton Laboratories 1989h).

*Chlorothymol*. Szybalski (1958) reported that Chlorothymol was nonmutagenic in the paper-disk method using *E. coli*.

*Thymol*. Thymol was tested for chromosome aberrations in *Allium* at a concentration from 0.01 to 0.00002 mol/L; the test was negative (Levan and Tjio 1948).

Nunn et al. (1971) investigated the arrest of mitosis by halothane (which contains 0.01% Thymol as a stabilizing additive). There was no significant difference in the total counts of normal metaphase, normal anaphase, intermediate and unclassifiable, and "colchicine" mitosis recorded with and without Thymol in the absence of halothane.

Fukuda et al. (1987) assessed the carcinogenic hazard of Thymol by examining SHE cells in culture. Induction of morphological transformation (MT), UDS, and SCEs in the cells following Thymol treatment (0.3 to 30 µg/ml) were considered markers for risk assessment.

Compared to dosing with 0.3 µg/ml, which produced a UDS of  $615.4 \pm 38.5$ , UDS was significantly increased in SHE cells when treated with 1, 3, or 10 µg/ml for 48 h to  $859.1 \pm 52.4$ ,  $725.3 \pm 39.5$ , and  $981.2 \pm 48.5$ , respectively. The amount of MT was increased significantly from control values of 0% transformations to 0.27%, 0.28%, and 0.34% at concentrations of 3, 10, and 30 µg/ml of Thymol, respectively. The number of SCEs increased significantly from the control value of  $8.85 \pm 2.86$  at Thymol concentrations of 0.3, 1, 3, 10, and 30 µg/ml to  $12.30 \pm 4.13$ ,  $11.82 \pm 3.95$ ,  $10.93 \pm 3.52$ ,  $12.34 \pm 4.33$ , and  $11.12 \pm 3.00$ , respectively (Fukuda et al. 1987).

Instituto Superiore di Sanità (1999) reported that growth of V79 cells was completely inhibited by 30 µg/L Thymol for 24 to 48 h. Cells exposed to Thymol at 30 to 300 µg/L for 2 h showed concentration dependent inhibition of DNA, RNA, and protein synthesis.

Stammati et al. (1999) performed a DNA repair test using Thymol against test strains of *E. coli* WP2 *trpE65* and its isogenic DNA repair-deficient derivative CM871 *trpE65*, *uvrA155*, *recA56*, *lexA*. The positive control was 4-nitroquinoline-*N*-oxide. Thymol was tested at concentrations of 2.5, 3, 5, and 6 µmol in triplicate. After an overnight incubation, the inhibition zones were measured and the differential killing between the tests strains was regarded as an indication of Thymol's ability to produce DNA damage. Thymol was only marginally more toxic to CM871 than to WP2 cells and is considered marginally toxic to DNA repair.

These authors also performed an SOS Chromotest using Thymol at doses of 0.0001, 0.001, 0.010, and 0.025 µmol. The test is based on the fusion of *lacZ*, the bacterial β-galactosidase gene, with the *sfIA* gene. The latter is one of the SOS genes, which are induced as a result of DNA damage in *E. coli*.

Only the highest dose was positive. The authors concluded that this probably reflects some secondary effect caused by the

toxicity of the compound rather than a real SOS induction and the genotoxic potential of Thymol is very weak (Stammati et al. 1999).

*o*-Cymen-5-ol. The mutagenicity of *o*-Cymen-5-ol was tested using the preincubation method with and without metabolic activation by S9. *E. coli* strain WP2uvrA was tested with 1 to 800  $\mu$ g/plate *o*-Cymen-5-ol. Positive and negative controls were used. *o*-Cymen-5-ol was negative in this assay (Osaka City Institute of Public Health and Environmental Sciences 1981).

*Carvacrol*. Stammati et al. (1999) performed a DNA repair test using Carvacrol using test strains of *E. coli* WP2 *trpE65* and its isogenic DNA repair-deficient derivative CM871 *trpE65*, *uvrA155*, *recA56*, *lexA*. The positive control was 4-nitroquinoline-*N*-oxide (4  $\mu$ g/disc). Carvacrol was tested at concentrations of 2.5, 3, 5, and 6  $\mu$ mol in triplicate. After an overnight incubation, the inhibition zones were measured and the differential killing between the tests strains was regarded as an indication of Carvacrol's ability to produce DNA damage.

Carvacrol was only marginally more toxic to CM871 than to WP2 and was considered marginally toxic to DNA repair.

These authors also performed an SOS Chromotest using Carvacrol at concentrations of 0.0001, 0.001, 0.010, and 0.025  $\mu$ mol. Only the highest dose was positive. The authors concluded that this probably reflects some secondary effect caused by the toxicity of the compound rather than a real SOS induction. The authors concluded that the genotoxic potential of Carvacrol in the test system is very weak although its action at DNA level cannot be excluded because it did cause nuclear fragmentation (Stammati et al. 1999).

## CARCINOGENICITY

*Thymol*. The ability of Thymol to induce primary lung tumors was investigated using mice (Stoner et al. 1973). Groups of 15 A/He mice per sex per dose received ip injections of Thymol three times a week for 8 weeks. The total Thymol dose was 1.20 or 6.00 g/kg/mouse. The vehicle was tricaprylin (0.1 ml). The mice were 6 to 8 weeks old and weighed 18 to 20 g. Mice were killed 24 weeks after the beginning of dosing. Survival, body weights, necropsy, and examination of liver, kidney, spleen, thymus, lungs, intestine, and salivary and endocrine glands were recorded. The positive control mice received 10 or 20 mg urethane. Eighty mice per sex served as controls (50 mice per sex were untreated controls and 30 received a water vehicle).

All 30 (15 male/15 female) mice treated with 6.00 g/kg Thymol survived. Twenty-nine of 30 mice treated with 1.20 g/kg Thymol survived. The tumor incidence for mice administered a total dose of 1.20 g/kg was 3/14 for female mice (1 female died) and 5/15 for male mice. The tumor incidence for mice administered a total dose of 6.00 g/kg was 2/15 for male and female mice. The negative control had 4/80 male mice deaths and 4/80 female mice deaths. The number of negative control mice

with lung tumors were 19/80 males and 20/80 females and the number of tumors per mouse averaged between  $0.17 \pm 0.02$  and  $0.37 \pm 0.07$ . The positive control had 0/40 male mice deaths and 1/40 female mice deaths. The percent of positive control mice with lung tumors was 100% and the number of tumors per mouse averaged between  $9.1 \pm 2.28$  and  $21.8 \pm 4.48$ . Thymol was negative for inducing primary lung tumors in mice (Stoner et al. 1973).

## Cocarcinogenicity

*m*-Cresol, *o*-Cresol, and *p*-Cresol. The incidence of skin tumors in albino mice was investigated using *m*-Cresol, *o*-Cresol, and *p*-Cresol. One drop (25  $\mu$ l) of each cresol isomer was applied to shaved skin twice weekly at 20% (in benzene) for 12 weeks. The initiator 9,10-dimethyl-1,2-benzanthracene (DMBA) was used at 0.3% (in acetone). *m*-Cresol induced an average number of 0.93 papilloma per mouse, *o*-Cresol induced an average of 1.35, and *p*-Cresol induced an average of 0.55. Only 14 of 29, 17 of 27, and 20 of 28 mice survived dosing with *m*-Cresol, *o*-Cresol, and *p*-Cresol, respectively. The 12 control mice developed no papillomas. No carcinomas were observed in any mice (Boutwell and Bosch 1959).

*o*-Cresol. Yanysheva et al. (1993) evaluated the effect of oral administration of benzo(a)pyrene and *o*-Cresol on female CC57Br mice (30 to 45 mice/dose group). Mice weighed 12 to 14 g and were dosed twice weekly (for a total of 10, 20, or 40 doses). The chemicals were administered in one of three ways: simultaneously *o*-Cresol and benzo(a)pyrene, *o*-Cresol then benzo(a)pyrene, or benzo(a)pyrene then *o*-Cresol. The doses were 1 or 5 mg of benzo(a)pyrene and 0.02, 1, or 10 mg of *o*-Cresol in triethylene glycol and administered as 0.1 ml water solutions. The experiments lasted from 5 weeks up to 30 weeks for the groups with the large chemical doses. All tumors greater than 1 mm in diameter were recorded. A negative control of 2 mg triethylene glycol was administered to mice 20 times over 10 weeks. A positive control of 5 mg benzo(a)pyrene was given to mice 10 times over 5 weeks and 1 mg benzo(a)pyrene was given 20 times over 10 weeks. *o*-Cresol was administered to mice 20 times in 10 weeks in doses of 0.02 or 1 mg with 2 mg of triethylene glycol. *o*-Cresol (1 mg) was also administered 20 times over 10 weeks to mice.

During simultaneous administration of *o*-Cresol (1 mg), and benzo(a)pyrene (1 mg), the incidence and multiplicity of tumors as well as the degree of malignancy increased, and the latency was shortened. At identical doses, when *o*-Cresol was administered before or after benzo(a)pyrene, the carcinogenic effect decreased. *o*-Cresol at 0.02 mg (with 2 mg triethylene glycol) did not modify carcinogenesis compared to negative controls (triethylene glycol). When *o*-Cresol (10 mg) and benzo(a)pyrene (5 mg) were administered simultaneously, the incidence of malignant tumors was similar to controls receiving benzo(a)pyrene only (13.8% of mice). The authors concluded that this was indicative of inhibition of carcinogenesis, which may be related to the toxic effects of *o*-Cresol (Yanysheva et al. 1993).

## Tumor Promotion

*m-Cresol*, *o-Cresol*, and *p-Cresol*. Boutwell (1967) reported that *m-Cresol*, *o-Cresol*, and *p-Cresol* have the same order of activity as phenol in terms of skin tumor promotion; no other specific data were available on the Cresols, but data were only provided for mice treated with phenol. Mice (strain not stated) were treated with a single topical application of the initiator, DMBA at a dose of 75  $\mu\text{g}$ . One week later the mice were treated with a topical application of 1.25 mg of 10% phenol (in benzene), 2.5 mg of 10% phenol (in benzene), or 5.0 mg of 20% phenol (in benzene) twice weekly for 35 weeks. The control groups received either phenol or DMBA.

A decreased response was obtained in the dose group receiving 1.25 mg phenol per mouse twice weekly. The 2.5-mg phenol dose group gave a maximal tumor response whereas the high dose group was fatal to some mice as a result of systemic toxicity. At the 2.5-mg phenol dose, mice treated with 10% phenol (in benzene) developed their first carcinomas at 19 weeks, at 26 weeks 50% of mice had carcinomas, and at 42 weeks 73% of mice had carcinomas. Few skin tumors developed on the mice treated with DMBA or phenol alone; there was only one fibrosarcoma at 58 weeks in the mice treated with phenol alone (Boutwell 1967).

*p-Cresol*. Predicated on *p-Cresol* as a microbial amino acid metabolite which has shown weak promoting activity in animal studies, Renwick et al. (1988) conducted a study to determine if *p-Cresol* (also indole and phenol) would act as a promoter of bladder cancer in humans. Twenty-two males and 10 females with histologically confirmed carcinoma of the urinary bladder were matched with similar controls by age and sex (however, weight and smoking habits could not be matched).

*p-Cresol* (also indole and phenol) excretion had wide interindividual variation, but there were no significant differences between the two groups. The mean urinary excretion of *p-Cresol* for controls was  $54.8 \pm 38.7$  mg/day and  $51.4 \pm 32.5$  mg/day for patients. The findings indicated that the "endogenous metabolites [*p-Cresol*] do not contribute significantly to the development of human bladder cancer" (Renwick et al. 1988).

## Antitumor Activity

*Thymol*. He et al. (1997) investigated the antitumor activity of Thymol using Murine B16 (F10) melanoma cells incubated with Thymol for 48 h. During a 48-h incubation, the mean concentration of Thymol needed to inhibit the increase of Murine B16 melanoma cells by 50% was calculated to be  $120 \pm 15$   $\mu\text{mol/L}$ .

*Carvacrol*. He et al. (1997) investigated the antitumor activity of Carvacrol using Murine B16 (F10) melanoma cells incubated with Carvacrol for 48 h. During a 48-h incubation, the mean concentration of Carvacrol needed to inhibit the increase of Murine B16 melanoma cells by 50% was calculated to be  $120 \pm 15$   $\mu\text{mol/L}$ . The additive effects of Carvacrol paired with  $\beta$ -ionone on the growth of B16 melanoma cells

in culture were also investigated. Carvacrol (50  $\mu\text{mol/L}$ ) and  $\beta$ -ionone (75  $\mu\text{mol/L}$ ) reduced the 48-h cell count by 25% and 31%, respectively; the additive effect was a 48% reduction in cell number, which was less than the predicted 56% reduction by sum of the individual effects. A similar effect was seen when the concentrations of Carvacrol and  $\beta$ -ionone were doubled.

Zeytinoğlu et al. (1998) investigated the antitumor activity of Carvacrol against lung tumors induced by DMBA in vivo. Adult Wistar rats (200 to 250 g, 4 animals/group) of both sexes were fed standard diet and water ad libitum. Carvacrol dissolved in olive oil was applied four times in 8 days. In order to induce lung tumors in vivo, DMBA was dissolved in sesame oil and applied once. Control groups received olive oil, sesame oil, and 0.9% NaCl alone; colchicine was used for standard antitumor agent 3 days in a week.

No tumors developed in the control groups; however, the color of the lungs were darker and not smooth in the group treated with olive oil and sesame oil. In the Carvacrol treated group, all the lungs were observed to be healthy as observed in the NaCl group. The lungs were transparent and had smooth margins, indicating complete loss of tumor development (Zeytinoğlu et al. 1998).

## CLINICAL ASSESSMENT OF SAFETY

### Dermal Irritation

*PCMC*. Patch testing with 2% PCMC in petrolatum may produce irritant reactions, particularly in people with multiple patch test reactions, that are misinterpreted as allergic responses (Lewis and Emmett 1987). Often, retesting several months after the dermatitis had cleared did not give positive results.

*o-Cresol*. *o-Cresol* was tested in a human maximization test using a 48-h closed patch test on the forearm of 25 volunteers (male and female). There were no effects at 4% *o-Cresol* in petrolatum (RIFM 1980b as cited in RIFM 2001c).

*p-Cresol*. *p-Cresol* was patch tested at a concentration of 4% in petrolatum on the backs of five male subjects. No effects were observed (RIFM 1972a as cited in RIFM 2001d).

*p-Cresol* was tested for dermal irritation at a concentration of 4% in petrolatum on 25 male subjects. No effects were observed (RIFM 1972a as cited in RIFM 2001d).

*Thymol*. A 48-h patch test was conducted using 4% Thymol in petrolatum on the backs of five male subjects. No effects were observed (RIFM 1972c as cited in RIFM 2001e).

Thymol was tested for dermal irritation at a concentration of 4% in petrolatum on 25 male subjects. No effects were observed (RIFM 1972c as cited in RIFM 2001e).

A 24-h patch test using Thymol was conducted on 25 subjects (12 male, 13 female). The vehicle and negative controls were Vaseline. Reactions were graded at 1 and 24 h after patch removal. Thymol was tested at 1% and 5% on the upper arms. There were no effects at 1%. No other details available (CTFA 1997a).

*o*-Cymen-5-ol. A 24-h patch test was conducted on 53 female volunteers to determine the skin irritating effects of *o*-Cymen-5-ol. The purity of *o*-Cymen-5-ol used for testing was >99%. To the forearm of each subject were applied three patches, one containing Vaseline, one containing 0.1% *o*-Cymen-5-ol in Vaseline, and another containing 1.0% *o*-Cymen-5-ol in Vaseline. Skin reactions were evaluated within 3 h following removal of the patches. No skin irritation was observed in any subject (CTFA 1980d).

*Carvacrol*. Carvacrol was patch tested at a concentration of 4% in petrolatum on the backs of 31 subjects. No effects were observed (RIFM 1977 as cited in RIFM 2001a).

### Dermal Sensitization

*PCMC*. A single occlusive patch of a below-irritation dose of PCMC (concentration not specified) was applied for 48 h to 363 patients with allergic contact dermatitis (Burry 1969). Upon scoring after 96 h, three patients had positive reactions to PCMC.

In a modified human Draize study using 31 male subjects, 5% PCMC in petrolatum was used for both induction and challenge (Marzulli and Maibach 1973). Induction consisted of 10 successive applications of 5% PCMC in petrolatum under occlusive patches being applied to the upper lateral portion of the arm; the patches were removed after 48 or 72 h. After a 2-week nontreatment period, a challenge patch of 5% PCMC in petrolatum was applied for 72 h. None of the 31 subjects were sensitized.

In another Draize test performed using groups of 98, 88, and 66 male subjects were induced with 5%, 10%, or 20% PCMC in petrolatum, respectively, for 3 to 5 weeks. Ten 48 to 72 h applications of 0.5 g of the test material were made under an occlusive patch to the upper lateral portion of each subject's arm. Following an approximately 2 week nontreatment period, subjects of all three groups were challenged with a 72-h patch containing 5% PCMC in petrolatum. None of the subjects in the three test groups responded to the challenge patch (Marzulli and Maibach 1974).

Two percent PCMC in petrolatum was added to the standard patch series in 1972 (Cronin 1980). During the time period of 1973–1976, the incidence of PCMC sensitivity varied between 0.2% and 1%, 0.3% and 0.6%, and 0.3% and 0.6% for men, women, and overall, respectively. The total incidence for this time period was 0.5% (17/3189), 0.4% (13/3630), and 0.4% (30/6819) for men, women, and overall, respectively.

Wilkinson et al. (1980) performed patch tests on patients in two regions of England using allergens included in a Standard Patch Test Battery, one of which was PCMC, due to concern regarding medicament sensitivity. A total of 651 patients, 267 men and 384 women, were tested in one region and 1029 patients, 373 men and 656 women, were tested in the other region. The occlusive patches were removed after 2 days and the test sites were again scored after 4 days. The dose was not given.

Of the 651 patients in the first region, a total of 0.8% had positive patch test results to PCMC; 0.5% of the men and 1.1%

of the women reacted to PCMC. In the second region, a total of 0.7% of the patients had positive reactions; 1.3% of the men and 0.3% of the women reacted to patch testing with PCMC. It was unclear why PCMC sensitivity varied between men and women.

An additional 45 patients, 11 men and 34 women, and 128 patients with leg ulcers and stasis eczema, 36 men and 92 women, were patch tested in the two regions. Of the 45 patients tested in the first region, a total of 4.4% had positive reactions to patch testing with PCMC; 9% of the men and 2.9% of the women reacted positively to PCMC. In the second region, 0.8% of the 128 patients reacted positively to PCMC; 0.0% of the men and 1.1% of the women reacted to patch testing with PCMC (Wilkinson et al. 1980).

Andersen and Hamann (1984a) tested consecutive eczema patients with the International Contact Dermatitis Research Group (ICDRG) standard patch test series, which included PCMC-containing biocides. Reactions were scored according to the recommendations of the ICDRG (Wilkinson et al. 1970). Of 1462 patients tested with 2% PCMC in petrolatum, only 5 had positive patch test results and 6 had irritant reactions; none of the positive results were clinically explainable.

Andersen and Veien (1985) tested consecutive eczema patients with the ICDRG standard series. PCMC, 1% in petrolatum, was tested on 671 patients and no positive reactions were observed. PCMC was withdrawn from the patch test battery after 3 months due to the lack of reactions observed.

*Thymol*. Meneghini et al. (1971) determined the incidence of acquired contact sensitivity in patients with eczematous dermatitis of different types using a 48-h occlusive patch on intact skin. Between 1967 and 1970, 290 patients were tested using 1% Thymol (in petrolatum). There was no reaction in the 290 patients. In a 48-h patch test in 50 normal people, the highest tolerated concentration of Thymol was 5% (in petrolatum).

Larsen (1977) performed standard 48-h patch tests using 1% Thymol (in petrolatum) on 20 perfume-sensitive male and female patients. Reactions were read at removal or 24 h after removal. Fifty control patients tested negative. One “perfume-sensitive” patient reacted to Thymol.

Berova (1990) reported on 84 contact dermatitis patients. All patients were patch tested with 17 different allergens in series. Thymol was tested since it is a common dental antiseptic. Thymol at a concentration of 1% in petrolatum was patch tested. Only one patient reported a positive reaction to Thymol.

*Carvacrol*. Calnan (1972) conducted tests using 1% Carvacrol in petrolatum on two patients with contact dermatitis. Positive effects were reported in one of two patients tested.

RIFM (1976b) reported that 33 subjects dermally exposed to 8% Carvacrol in petrolatum exhibited no adverse reactions.

RIFM (1977) reported that 31 subjects exposed dermally to 4% Carvacrol in petrolatum exhibited no adverse reactions.

DeGroot et al. (1985) patch-tested patients with 5% Carvacrol in petrolatum. There were 179 patients tested with suspected cosmetic allergies (144 women, 34 men, and 1 person's sex was not recorded). Reactions were read after 48 and 72 h. There

were sensitization effects recorded, and two reactions may have been false positives due to Excited Skin Syndrome. No other information was available.

Meynadier et al. (1986) patch-tested 28 patients allergic to perfumes and sweet-smelling constituents using 2% Carvacrol in petrolatum. Positive reactions were observed in 3 of 28 patients.

*o*-Cymen-5-ol. CTFA (1978) reported a maximization test conducted on 27 men to determine the ability of *o*-Cymen-5-ol to induce skin sensitization. The procedure used was a modification of the method described by Kligman (1966). The five materials evaluated were Vaseline (vehicle control), *o*-Cymen-5-ol in Vaseline, a cream base (vehicle control), *o*-Cymen-5-ol in cream base, and petrolatum (negative control). For each test material, a total of five 48-h induction patches were applied to the forearm at the same site. The initial induction exposure was preceded by a 24-h occlusive patch containing 5% aqueous sodium lauryl sulfate (SLS). *o*-Cymen-5-ol concentrations employed during the induction phase were 1.0%. Ten to 14 days after the induction phase, challenge patches were applied to previously untreated sites for 48-h. Challenge exposures were preceded by 30 min applications of 5% aqueous SLS under occlusion to the left-hand side of the back. Challenge patches were also applied without SLS pretreatment to the right-hand side of the back. The concentration of *o*-Cymen-5-ol employed during the challenge phase was 0.1%.

On SLS-pretreated sites, the authors described low-grade irritant reactions in several individuals reacting to each of the five substances at the 48-h challenge reading. By the 72-h reading, approximately half the subjects had low-grade irritant reactions to each of the five test materials. On sites receiving no SLS treatment, no reactions were observed to any test material at either the 48 or 72-h challenge reading. It was concluded that *o*-Cymen-5-ol produced no reactions that could be considered irritant or allergic in nature (CTFA 1978).

TKL Research Inc. (1992) performed a human repeated insult patch test (RIPT) to assess the skin sensitization of 0.1% *o*-Cymen-5-ol. Ninety-nine subjects (aged between 20 and 77 years) completed the study over 6 weeks. The test material (0.2 ml) was dispensed onto the occlusive patch and applied to the back. After 24 h, the patch was removed. This process was repeated for a series of nine 24-h exposures. Reactions were recorded after 72 h. Fourteen days after the last exposure, subjects were retested at the same dose and reactions were scored after 24 and 48 h post application. One patient had an "aquamarine" taste and experienced transient nausea after the third patch application. The sensitization disappeared 2 days after discontinuance from the study; the relationship to the product could not be determined. There was no evidence of skin sensitization from 0.1% *o*-Cymen-5-ol exposure during the course of the study.

*m*-Cresol. Seidenari et al. (1991) patch-tested adult patients that were sensitized to textile dyes for reaction to *m*-Cresol using Finn chambers and Scanpor tape. There were positive effects in 2 of 81 patients tested with 2% *m*-Cresol.

*o*-Cresol. RIFM (1980b) reported no effects in 25 subjects tested with 4% *o*-Cresol for sensitization effects.

*o*-Cresol/*p*-Cresol. Bruze and Zimerson (1997) conducted closed 2-day patch tests using 81% *o*-Cresol or *p*-Cresol (in ethanol) on 10 hand dermatitis patients who had previously reacted to phenol-formaldehyde resins and at least 1 methylol phenol compound. Reactions were graded on day 3 and patients that reacted were retested with 10-fold dilutions until a negative response was obtained. Four patients tested positive at 81% *o*-Cresol, three tested positive at 8.1%, and there were no effects at 0.81% *o*-Cresol. One patient tested positive at 81% *p*-Cresol and there were no effects at 8.1% *p*-Cresol.

*Chlorothymol*. The AMA Laboratories (1996) performed a human RIPT to assess the skin irritation and sensitization of an OTC topical cream containing 0.032% Chlorothymol. One hundred ten subjects completed the study, 93 were female, and 18 were male. Subjects were aged between 16 and 77 years. The test material (0.2 ml) was dispensed onto the semioclusive patch and applied to the back. After 24 h the patch was removed. This process was repeated for a series of nine 24 h exposures were made every Monday, Wednesday, and Friday for 3 weeks. Reactions were recorded prior to each patch application. Ten to 14 days after the last exposure, subjects were retested at the same dose and reactions were scored after 24 and 48 h. No adverse reactions were noted during the course of the study, and the OTC topical cream containing 0.032% Chlorothymol under semioclusion was considered a nonprimary irritant and a nonprimary sensitizer.

### Cross-Reactivity

Cross-reactivity has been observed between PCMC and chloroxylenol (Hjorth and Trolle-Lassen 1963). However, the cross-reactivity has only occurred where the initial sensitization was to chloroxylenol, not to PCMC (Lewis and Emmett 1987).

As reported by Smeenk et al. (1987) and the Instituto Superiore di Sanità (1999), 13 patients (over an eleven year period) sought treatment in a Dermatology Department for a contact allergy to Hirudoid cream. The separate ingredients gave negative results in patch tests. The simultaneous presence of the preservatives 1,3,5-trihydroxyethylhexahydrotriazine and Thymol was found to be necessary for the occurrence of a positive patch test where both ingredients were used as preservatives. Thymol reacted with the degradation products of a triazine derivative in the heparinoid cream to cause the contact allergy.

### Chemical Leukoderma

Mathias (1988) listed *p*-Cresol and many other *p*-substituted phenols as a common cause of chemical leukoderma (toxic vitiligo). Lesions were reportedly widespread in areas involving direct skin contact and systemic absorption by accidental ingestion or percutaneous absorption which has been postulated to explain lesions on skin that had not been exposed to the chemical.

## Photoallergy

Twenty-five women were induced via six occlusive patches (1% *p*-Cresol in a hydrophilic ointment) each followed by 3-MED (minimal erythema dose) irradiation. Challenge was 10 days later via an occlusive patch followed by 4 Joules/cm<sup>2</sup>. No photoallergic reactions were noted. There were 11 allergic contact sensitivity reactions and 7 primary irritation reactions (RIFM 1982 as cited in RIFM 2001d).

## Other Toxicity

Syrowadko and Malysheva (1977) reported that women exposed in their workplace (enamel-insulated wire manufacturing) to varnishes that contained Mixed Cresols (amount not stated) had increased gynecological problems such as menstrual disorders and hormonal disturbances. An increased frequency of perinatal mortality and abnormal development of newborn infants was also reported.

Mixed Cresols can cause hemolytic anemia in patients with apparently normal erythrocytes (Chan et al. 1982; Chan et al. 1971; Cote et al. 1984; Lee et al. 1993).

*o*-Cresol is a respiratory irritant in humans. Eight of 10 subjects exposed to "brief exposure" to a concentrated aerosol of 6 mg/m<sup>3</sup> *o*-Cresol complained of dryness, nasal constriction, and throat irritation (U.S. Department of Health and Human Services 1992).

## Medical Treatment

*o*-Cymen-5-ol has been shown to be effective in treating a number of dermatological diseases in Japan (Jan Dekker International 1993). *o*-Cymen-5-ol was administered as a 3% ointment either alone or in conjunction with some method of supportive therapy. Occasionally, miscellaneous side effects were reported. It was noted that careful attention should be paid to the use of *o*-Cymen-5-ol solution on persons with delicate skin, especially infants. Table 10 summarizes the use of 3% *o*-Cymen-5-ol in treating dermatological diseases.

## Case Reports

PCMC is a strong sensitizer in guinea pig maximization tests, but human sensitization has been reported to be fairly rare (Lewis and Emmett 1987). For other cresols, a number of case studies were reported in the published literature and are summarized in Table 11.

## Occupational Exposure and Limits

Exxon Research and Engineering Company (1983) obtained urine samples from process technicians in a coal liquefaction plant where *m*-Cresol, *o*-Cresol, and *p*-Cresol are minor constituents of several of the process streams. All samples were below the detection limit of 0.03 ppm.

Substantial amounts of Cresols are produced or imported into the United States each year. It was estimated that 132.4 million pounds of Cresols were produced or imported in 1984 and

**TABLE 10**

Use of 3% *o*-Cymen-5-ol in treating dermatological diseases  
(Jan Dekker Intl. 1993)

Disease	No. of cases	Effectiveness
Folliculitis	8	Markedly effective- 5 Effective- 3
<i>Staphylococcal</i> impetigo	10	Markedly effective- 2 Effective- 8
<i>Streptococcal</i> impetigo	2	Markedly effective- 2
Bockhart's impetigo	3	Markedly effective- 3
Impetiginous eczema	3	Markedly effective- 3
Tinea capitis	2	Markedly effective- 1 Effective-1
Athlete's foot	52	Markedly effective- 16 Effective- 29 Ineffective- 7
Superficial ringworm of the scalp	4	Markedly effective- 2 Effective- 2
Vesiculomacular ringworm	33	Markedly effective- 14 Effective- 16 Ineffective- 3
Ringworm of the nails	4	Markedly effective-1 Effective- 3
Tinea cruris	21	Markedly effective- 7 Effective- 10 Ineffective- 4
Pityriasis rubra	2	Markedly effective- 2
Totals	144	Markedly effective- 38.9% Effective- 48.6% Ineffective- 12.95%

148,000 to 300,000 people were exposed via manufacturing, processing, and/or use activities (EPA 1986).

As reported by the NTP (1992d), the United States Environmental Protection Agency (USEPA) used a threshold level value (TLV) of 10 mg/m<sup>3</sup> to derive a chronic inhalation exposure limit of 7.143 mg/day (assuming a human breathing volume of 10 m<sup>3</sup>/day, a 5-day work week, and using an uncertainty factor of 10 to protect more sensitive individuals in a population).

According to the WHO (1995), exposure to Cresols can occur through air, water, or food. The median air concentration of *o*-Cresol was 0.359 ppb for 32 source-dominated sites in the United States.

According to Chou et al. (1998), the Agency for Toxic Substances and Disease Registry (ATSDR) developed a list of hazardous substances that included *m*-Cresol, *o*-Cresol, and *p*-Cresol. The ATSDR minimal risk levels (MRLs) were developed to provide screening levels for health assessors to identify contaminants and potential health effects that may be of

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**TABLE 11**

Case reports concerning substances that contain ingredients in the cresol family

Test material	Patient	Case	Testing/results	Reference
Sorbolene cream containing PCMC	40-year-old male	Severe hand and face dermatitis while using a sorbolene cream.	Patch testing strongly positive to PCMC and sorbolene cream after twice daily applications after 1 day.	MacKenzie-Wood and Freeman 1997
Steroid cream containing PCMC	39-year-old male	Dermatitis recurred on previously affected sites on the ankle and feet due to treatment with the steroid cream. An erythematous reaction with fine vesiculation was observed on the legs.	Patch testing with 1% PCMC resulted in ++ reactions after 48, 72, and 96 h. Subsequent testing several months later resulted in +++ reactions with 1 and 5%, ++ reaction to 0.1%, and + reaction to 0.01% PCMC after 96 h.	Lewis and Emmett 1987
Steroid cream containing PCMC	24-year-old male	Application of the steroid cream exacerbated existing dermatitis.	Patch testing with 2% PCMC resulted in ++ and +++ reactions after 48 and 96 h, respectively.	Oleffe et al. 1979
Steroid cream containing PCMC	40-year-old female	Application of the steroid cream caused existing eczema to deteriorate.	Patch testing with 2% PCMC (from two sources) resulted in + and ++ reactions and in - and ++ reactions after 48 and 96 h, respectively.	Oleffe et al. 1979
Glue and steroid cream containing PCMC	27-year-old female	Handling of the glue caused severe contact eczema; treatment with the steroid cream did not relieve the condition.	Patch testing with 2% PCMC was positive.	Dooms-Goossens et al. 1981
Steroid cream containing PCMC	18-year-old female	An eczematous eruption was treated with the cream for 6 mos; the condition worsened and spread.	Initial patch testing with 2% PCMC was negative. Patch testing after the condition worsened resulted in + reactions to 2% PCMC after 48 and 96 h.	Archer and MacDonald 1984
Steroid cream containing PCMC	35-year-old female	Treatment with the steroid cream for acute dermatitis initially resulted in transient improvement but then caused it to spread.	Patch testing with 2% PCMC resulted in ++ reactions after 48 and 96 h.	Goncalo et al. 1987
PCMC as a preservative in betamethasone cream	56-year-old female	Daily applications of dilute cream were being used to treat widespread dermatitis; application was stopped and the dermatitis cleared.	A patch test with PCMC was positive. (Previous testing gave positive reactions to chloroxylonol.)	Burry et al. 1975
PCMC as a preservative in betamethasone cream	35-year-old female	An acute contact dermatitis was treated with the cream; itching and mild dermatitis continued and mild contact dermatitis appeared elsewhere; this cleared with cessation of cream usage.	Patch tests with PCMC were positive. (Previous testing gave positive reactions to chloroxylonol.)	Burry et al. 1975

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**TABLE 11**Case reports concerning substances that contain ingredients in the cresol family (*Continued*)

Test Material	Patient	Case	Testing/Results	Reference
PCMC as a preservative in betamethasone cream	32-year old male	The cream was used to treat dermatitis; the dermatitis cleared once treatment was stopped.	A patch test reaction to 5% PCMC in soft paraffin was greater than the reaction to 1% PCMC in soft paraffin. (Positive reaction to chloroxylenol was slight.)	Burry et al. 1975
PCMC as a preservative in betamethasone cream	43-year-old female	The cream was used to treat dermatitis; mild contact dermatitis appeared elsewhere. The dermatitis cleared once treatment was stopped.	Upon patch testing, there was no reaction to 1 or 5% PCMC in soft paraffin. (A strong positive reaction to chloroxylenol was observed.)	Burry et al. 1975
PCMC, 0.15%, as a preservative in mucous heparin	35-year-old woman	The patient was given 10,000 units of the heparin IV and immediately collapsed after the second dose with pallor, sweating, hypotension, and tachycardia. She recovered spontaneously after 30 min.	Intradermal skin tests gave positive reactions to both PCMC-preserved and preservative-free heparin.	Hancock and Naysmith 1975
PCMC, 0.15%, as a preservative in mucous heparin	55-year-old man	The patient was given 10,000 units of the heparin IV and over the next hour developed nasal congestion, profuse sweating, and a generalized urticarial rash.	Intradermal skin testing with preservative-free heparin did not produce a reaction.	Hancock and Naysmith 1975
PCMC, 0.15%, as a preservative in mucous heparin	Seven patients (sex unspecified)	The patients were given 10,000 units prophylactic subcutaneous heparin twice daily. Within a few hours after the first and subsequent injections, an indurated erythematous reaction developed at the injection site.	Intradermal skin testing with preservative-free heparin in four of these patients produced no response.	Hancock and Naysmith 1975
PCMC, 0.15%, as a preservative in mucous heparin	21-year-old woman	The patient was given 5000 units heparin 6-hourly. Administration of the first two doses resulted in severe burning pain at the injection site, radiating along the veins and up the forearm into the arm, nausea, light-headedness, and drowsiness with pallor and sweating.	A dose of PCMC-free heparin did not result in the pain of a systemic reaction. Formal intradermal skin testing produced a reaction to the mucous heparin but none to preservative-free heparin.	Ainley et al. 1977
Cleaning detergent disinfectant containing PCMC	28-year-old woman	The patient complained of red swollen eyelids each time she used the detergent disinfectant [of which the two active principles are PCMC and <i>o</i> -benzyl- <i>p</i> -chlorophenol (OBPCP)].	PCMC was diluted to 1 and 10% in water:ethanol (1:1) and tested with open and prick tests. The results of the open test were negative and +++ for 1 and 10%, respectively, and of the prick test were +? and +++ for 1 and 10%, respectively.	Freitas and Brandao 1986

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**TABLE 11**Case reports concerning substances that contain ingredients in the cresol family (*Continued*)

Test Material	Patient	Case	Testing/Results	Reference
Mixed Cresols	NA	Cresol ingestion.	Open tests for 30 min with the detergent at 20% in water elicited an urticarial wheal. After the wheal, tests with PCMC produced superficial necrosis, which could be observed on the eyelids at the same time. Testing with the detergent at 10% in water and of OBPCP were negative. Can produce hemolysis that causes hemoglobinuric tubular necrosis usually in conjunction with hypotension.	Cason 1959; Muehrcke 1969
Mixed Cresols (50% solution)	37-year-old woman	Swallowed about 250 ml of a disinfectant described as 50% Mixed Cresols in a mixture of linseed oil, potassium hydroxide, and water.	Death was caused by acute intravascular hemolysis, which resulted in multiple thrombosis and renal failure. Another woman consumed 100 ml of the same disinfectant and recovered.	Chan et al. 1971
Cresol mixture	Woman	Swallowed between 500 and 750 ml of a concentrated Cresol mixture.	Died from cardiac arrest after 26 h. Tachycardia with polymorphic ventricular extrasystoles were noted shortly after exposure. Diffuse necrosis of the bronchial epithelium was noted and thought to have occurred prior to death. Edema and hemorrhage were also observed.	Labram and Gervais 1968
Cresol derivative (90% Mixed Cresols in water)	1-year-old male infant	A 1-year-old infant had 20 ml of a Cresol derivative spilled on his head and covered roughly 7% of his body surface.	The infant went into a coma and died within 4 h. Assuming the infant weighed approximately 10 kg, the lethal dose was estimated to be about 2 g/kg. Cresols were identified in the blood (12 mg/100 ml), liver, and brain.	Green 1975
Cresol (50%) soap solution	44-year-old man	A 44-year-old man was found unconscious after ingesting 300 ml of 50% cresol-soap solution.	Endotracheal intubation, gastric lavage, and activated charcoal were administered. He had dermal burns, esophageal and gastric erosion, pneumonia, mixed metabolic acidosis, and hemolysis developed. Although the amount ingested exceeded the reported lethal dose of 30 to 60 grams, the patient recovered.	Wu et al. 1998

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**TABLE 11**Case reports concerning substances that contain ingredients in the cresol family (*Continued*)

Test Material	Patient	Case	Testing/Results	Reference
Cresol	50-year-old man	A 50-year-old man drank a Cresol solution. He was found unconscious and sent to the hospital.	He manifested the signs of typical cyanosis with methemoglobinemia and his urine showed high amounts of Cresol and phenol. The contents of met-form hemoglobin increased in the patient's blood drastically after 15 h, an exchange transfusion was done and the levels of these hemoglobins decreased extensively and the patient recovered.	Minami et al. 1989
Cresol (50%)	26-year-old woman	A 26-year-old woman was treated 8 h after ingesting 70 ml of 50% Cresol.	Marked increases of aminotransferases were noted roughly 24 h after ingestion. The patient recovered without any significant complications. Cresol and/or its metabolites may have caused transient hepato-cellular injury in the woman; this injury can manifest even after a 24-hour asymptomatic period. After 3 months all the woman's lab results were within normal limits.	Hashimoto et al. 1998
Cresol	Two female patients, 23- and 62-years-old	Two female patients, 23 and 62 years old, ingested Cresol.	Two female patients, 23- and 62-years-old, died of acute oral Cresol poisoning after 60 h and 90 h, respectively. There was characteristic zonal necrosis around the hepatic venules in both cases. The study focused on the liver, therefore no other information was available regarding other toxic effects from Cresol exposure.	Nakamura and Takahashi 1998
Listerine Antiseptic Solution (0.6% Thymol, 0.9% eucalyptol, 0.6% methyl salicylate, 0.4% menthol, and trace amounts of benzoic acid)	43-year-old man	A 43-year-old man applied Listerine Antiseptic Solution under occlusion for 3 weeks to a chronic paronychia of the big left toe which caused a spreading pruritic dermatitis.	A 48-h closed patch test performed, was positive in the patient and negative in 3 controls. In the patient and 3 control subjects there were no reactions in 48-h patch tests of 1% eucalyptus oil in alcohol, 2% methyl salicylate in olive oil, 1% menthol in petrolatum, and 5% benzoic acid in petrolatum. In a 48-h patch test using 1% Thymol in petrolatum all controls had no reaction, however the patient had a +2 reaction. The patient sensitized to Thymol was able to use it orally without any reaction. The author	Fisher 1989

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**TABLE 11**Case reports concerning substances that contain ingredients in the cresol family (*Continued*)

Test Material	Patient	Case	Testing/Results	Reference
Thymol (4%)	70-year-old woman	A 70-year-old woman had chronic paronychia of the middle and ring fingers of the right hand for 6 months prior to treatment with a 4% Thymol solution in chloroform applied once daily on affected proximal nail folds. Six weeks after beginning treatment, she developed severe erythema and edema with intense itching. A topical corticosteroid and discontinuation of Thymol use resolved the paronychia.	concluded that Thymol is a weak sensitizer that needs prolonged contact with occluded, inflamed skin, such as the paronychia area, to produce sensitization and dermatitis. One month later she was patch tested using the 4% Thymol solution and tested positive at day 2 and 3. A positive reaction to 1% Thymol in petrolatum was also noted at day 3. Twenty subjects control patch tested had no reaction to 1% Thymol in petrolatum. It was concluded that the 4% Thymol solution caused sensitization without any occlusion because it could more easily penetrate the proximal nail fold in their patient with chronic paronychia.	Lorenzi et al. 1995
Mouthwash containing Thymol	Three patients	Thyroid intoxication were reported from the use of a Thymol containing mouthwash. The patients had used mouthwash for 6 months to 3 years.	Body weight changes, clinical signs, systemic effects, and thyroid intoxication were noted in all patients. Two patients had lost weight, one exhibited tremors, restlessness, sleeplessness, languidness, palpitations, perspiration and diarrhea. All 3 patients recovered.	RIFM 2001e.
Thymol and Carvacrol	51-year-old man	A 51-year-old man, after exposure to wood for 2 years in a saw mill, developed acute dermatitis of the face, hands, and arms. Avoiding contact with wood cleared the symptoms.	The man was patch tested with 19 wood species of which western red cedar gave the strongest reactions (+3). The man was patch tested with patches containing 0.010 to 0.015 ml of Carvacrol or Thymol (= 0.10–0.15 mg of material) showed no reaction up to 96 h. Ten control subjects showed no reaction to Carvacrol and Thymol patch tests. Two other patients were patch tested that had +4 reactions to 0.1% and 1% thymoquinone. These two patients were also patch tested with 0.1% and 1% Thymol and Carvacrol. Neither patient had a reaction to 0.1% or 1% Thymol. However, one patient did have a +2 reaction to 0.1% and 1% Carvacrol.	Bleumink et al. 1973

concern at hazardous waste sites. An MRL is an estimate of the daily human exposure to a hazardous substance that is likely to be without an appreciable risk of adverse noncancer health effects over an acute (1 to 14 days), intermediate (15 to 364 days), and chronic (1 year or longer) exposure. The *m*-Cresol, *o*-Cresol, and *p*-Cresol MRLs for acute oral exposure with a respiratory end point were 0.05 mg/kg/day for all three isomers.

The permissible exposure limits (PELs) to Cresol isomers promulgated by the Occupational Safety and Health Administration is 5 ppm or 22 mg/m<sup>3</sup> in air (OSHA 2001). The ACGIH and the National Institute for Occupational Safety and Health (NIOSH) have recommended time-weighted average (TWA) threshold limit values of 5 ppm or 22 mg/m<sup>3</sup> in air; the Cresols do have a skin notation, which means there is the potential for significant exposure through the skin. All 14 countries listed in ILP Occupational Exposure Limits for Airborne Toxic Substances as of 1991 have set an occupational concentration of 5 ppm for TWA exposure for all Cresol isomers (WHO 1995).

## SUMMARY

Sodium *p*-Chloro-*m*-Cresol, *p*-Chloro-*m*-Cresol (PCMC), Mixed Cresols, *m*-Cresol, *o*-Cresol, *p*-Cresol, Isopropyl Cresols, Thymol, Chlorothymol, *o*-Cymen-5-ol, and Carvacrol are substituted phenols that are primarily used as cosmetic biocides/preservatives, but may be used as fragrance ingredients.

According to FDA (2001) and CTFA (2002) data, only PCMC, Thymol, and *o*-Cymen-5-ol are currently in use. The highest reported concentration of use was 0.5% *o*-Cymen-5-ol in perfumes.

Internationally, PCMC is approved for use in cosmetics by members of the European Union as a preservative at a maximum concentration of 0.2%; however, it may also be added to cosmetic products at a concentration other than that specified for a preservative for other specific purposes apparent from the presentation of the product. PCMC is prohibited in Europe for use in products intended to come in contact with mucous membranes. The Japanese Ministry of Health, Labor, and Welfare (MHLW) have put restrictions on Cresol, Thymol, and PCMC when used as a preservative in cosmetics. As preservatives, Cresol (rinse-off and leave-on products), Thymol (rinse-off and leave-on products), and PCMC (all products) are limited to 0.010, 0.05, and 0.50 g per 100 g, respectively. Cresol and Thymol are not permitted for use in cosmetics that are applied on mucous membranes (except Thymol when limited to the mouth).

In Japan, Cresol, Thymol, and PCMC are considered quasidugs; Cresol is required to be labeled appropriately in products used directly or indirectly on the body. Thymol and PCMC (as quasidugs) require appropriate labeling in products used directly on the body.

PCMC is approved for use as an indirect food additive in the USA. Many of the noncosmetic uses of PCMC utilize its anti-

septic properties. The use concentration for most non-cosmetic applications of PCMC ranges from 0.05% to 0.5%.

Cresols can be absorbed through skin, the respiratory tract, and the digestive tract and can penetrate into tissues. After absorption, most of the chemical is metabolized by the liver and either the metabolites or the unchanged chemical are excreted by the kidney with trace amounts excreted via the lungs. Cresol isomers are conjugated and excreted as glucuronides and sulfates. Although significant amounts of Cresols are secreted in the bile and undergo enterohepatic recirculation, urinary excretion is the main route for removing Cresols.

PCMC, *m*-Cresol, *o*-Cresol, *p*-Cresol, Thymol, *o*-Cymen-5-ol, and Carvacrol demonstrated antimicrobial activity against bacteria, yeast, and fungi. *m*-Cresol, *o*-Cresol, *p*-Cresol, Thymol, and Carvacrol have penetration enhancement effects in vivo and in vitro.

The following LD<sub>50</sub> values are a range for ingredients in the Sodium *p*-Chloro-*m*-Cresol family. In mice exposed orally, the lowest reported LD<sub>50</sub> was for *o*-Cresol and *p*-Cresol (344 mg/kg) and the highest LD<sub>50</sub> was for Thymol (1800 mg/kg). In rats exposed orally, the lowest reported LD<sub>50</sub> was for *p*-Cresol (207 mg/kg) and the highest LD<sub>50</sub> was for PCMC (5129 mg/kg). In guinea pigs exposed orally, the only reported LD<sub>50</sub> was for Thymol (880 mg/kg). In rabbits exposed orally, the lowest reported LD<sub>50</sub> was for Carvacrol (100 mg/kg) and the highest reported LD<sub>50</sub> was for *m*-Cresol (≥1400 mg/kg).

The only reported LD<sub>50</sub> for cats exposed orally was for Thymol (250 mg/kg).

In rats exposed dermally, the lowest LD<sub>50</sub> reported was for *p*-Cresol (750 mg/kg) and the highest LD<sub>50</sub> was for *m*-Cresol (1100 mg/kg). In rabbits exposed dermally the lowest LD<sub>50</sub> was for *m*-Cresol (1.80 ml/kg) and the highest LD<sub>50</sub> was Carvacrol (>5000 mg/kg). In rats exposed by inhalation, the lowest LC<sub>50</sub> reported was for *o*-Cresol (>20 mg/m<sup>3</sup>) and the highest LC<sub>50</sub> was for PCMC (>583 mg/m<sup>3</sup>).

In mice exposed orally the only LC<sub>50</sub> reported was for *o*-Cresol (178 mg/m<sup>3</sup>). In rats exposed percutaneously, the only LD<sub>50</sub> value reported was for PCMC (>500 mg/kg) and the only LD<sub>50</sub> value reported in mice was for PCMC (360 mg/kg). In mice exposed intravenously, the lowest reported LD<sub>50</sub> was for PCMC (70 mg/kg) and the highest LD<sub>50</sub> was for Thymol (100 mg/kg).

In rabbits exposed intravenously, the only reported LD<sub>50</sub> was for Thymol (60 mg/kg) and in dogs the only reported LD<sub>50</sub> was for Thymol (150 mg/kg).

In rats exposed subcutaneously, the lowest reported LD<sub>50</sub> was for PCMC (400 mg/kg) and the highest LD<sub>50</sub> was for Thymol (1600 mg/kg). In mice exposed subcutaneously, the lowest reported LD<sub>50</sub> was for Thymol (243 mg/kg) and the highest LD<sub>50</sub> was for Thymol (800 mg/kg). In mice exposed intraperitoneally, the lowest reported LD<sub>50</sub> was for Carvacrol (73.3 mg/kg) and the highest LD<sub>50</sub> was for *m*-Cresol (450 mg/kg).

In 28-day studies in rats and mice, an *o*-Cresol, *m*-Cresol, *p*-Cresol or *m*-Cresol/*p*-Cresol mixture in the diet produced a generally similar pattern of toxicities in both species. Dietary

concentrations of 3000 mg/kg appeared to be minimal effect levels for increases in liver and kidney weights and deficits in liver function. Histopathologic changes, including bone marrow hypocellularity, irritation to the gastrointestinal tract and nasal epithelia, and atrophy of female reproductive organs, occasionally occurred at 10,000 ppm, but were more common at the high concentration of 30,000 ppm.

Twenty-eight-day feeding studies conducted using mink or ferrets by administering *o*-Cresol in the diet resulted in a NOEL of 240 and 778 mg/kg, respectively. In two short-term oral toxicity studies, no significant dose-related toxicity (excluding body weight parameters) was observed when rats were given  $\leq 400$  mg/kg/day PCMC by gavage or  $\leq 10,000$  ppm PCMC in feed. In a short-term feeding study, groups of 5 to 10 male and female rats were fed a powdered diet containing 2% BHA or 2% *p*-Cresol. In contrast to BHA, *p*-Cresol did not induce forestomach lesions. In a short-term dermal toxicity study,  $\leq 160$  mg/kg PCMC was found to produce irritation and erythema, but had no systemic effects on rabbits.

Female mice exhibited no immunotoxicity when given *o*-Cresol ad libitum in drinking water at calculated doses of 0, 6.5, 32.5, 65, or 130 mg/kg/day for 14 days.

*m*-Cresol, *o*-Cresol, and *p*-Cresol effects on mouse hair pigmentation were studied over 6 months and demonstrated no effects for *m*-Cresol and *o*-Cresol. Only *p*-Cresol caused loss of pigmentation and at 0.5%, hair depigmentation was seen in black and agouti mice at 2 weeks, 4 weeks, and 6 months. In agouti mice, the tip of the hair was unaffected by *p*-Cresol. In the black, male mice, patches of hair pigment loss were induced by topical application of *p*-Cresol on the tip and hair shaft; depigmentation of the epidermis was also observed, especially on the tail. *p*-Cresol was toxic when large amounts (exact amount not stated) were painted on the skin. Repeated application to black mice had a local caustic and erosive effect, which was not observed in the agouti mice.

Rats exposed to  $2.1 \times 10^{-8}$  M Carvacrol for 4 or 8 weeks had moderate to dense selective degeneration in the olfactory bulbs.

No deaths were recorded in mice exposed to a mixture of *o*-Cresol aerosol and vapor for 1 month (2 h/day for 6 days a week) with an average concentration of 50 mg/m<sup>3</sup>. Cats exposed to 9 to 50 mg/m<sup>3</sup> of *o*-Cresol for 2 to 6 h/day for 1 month or more were reported to have inflammation and irritation of the upper respiratory tract, pulmonary edema, and hemorrhage and perivascular sclerosis in the lungs.

In short-term subcutaneous toxicity studies, 72 mg/kg and 0.25% PCMC did not have an effect on rats and rabbits, respectively.

In a subchronic study in which rats were fed  $\leq 1500$  ppm PCMC, the only observations made were retardation of body weights. In a 13-week toxicity study conducted by the NTP, 0, 1880, 3750, 7500, 15,000 and 30,000 mg/kg *o*-Cresol or *m*-Cresol/*p*-Cresol were added to the diet of rats and 0, 1250, 5000, 10,000, and 20,000 mg/kg *o*-Cresol and 0, 625, 1250, 2500, 5000, and 10,000 mg/kg *m*-Cresol/*p*-Cresol were added

to the diet of mice. The Cresol isomers produced a generally similar pattern of toxicities in rats and mice. A NOAEL in rats of 3750 ppm diet was calculated for *o*-Cresol. However, for *m*-Cresol/*p*-Cresol mixture the lowest dose tested resulted in changes in clinical chemistry and hyperplasia, therefore a threshold dose could not be established in rats. A NOAEL in mice of 1250 ppm and 625 ppm can be calculated for mice exposed to *o*-Cresol and *m*-Cresol/*p*-Cresol, respectively.

In a 13-week study, *m*-Cresol was administered by gavage at 0, 50, 150, or 450 mg/kg/day to rats. *o*-Cresol and *p*-Cresol were administered by gavage at 0, 50, 175, or 600 mg/kg/day. In the high-dose group, one male rat exposed to *m*-Cresol died, 19/28 females and 9/28 males exposed to *o*-Cresol died, and 3 high-dose females exposed to *p*-Cresol died. Clinical chemistry, hematology, and urinalyses parameters were not affected by *m*-Cresol, *o*-Cresol, or *p*-Cresol treatment. There was no evidence of gross or histopathologic lesions in rats of any *m*-Cresol or *o*-Cresol dose groups. Signs of central nervous system depression were observed in high-dose rats. *p*-Cresol was hepatotoxic at high doses to female rats, nephrotoxic at high doses to male rats, and induced a mild anemia in mid and high dose female rats. The study concluded 175 mg/kg/day of *o*-Cresol and 50 mg/kg/day of *p*-Cresol appeared to be the level at which no toxic effects were observed in rats.

Hamsters dosed with 1.5% *p*-Cresol in diet for 20 weeks had a greater incidence of mild and moderate forestomach hyperplasia as compared to the control. The labeling index in pyloric regions of the glandular stomach was slightly increased as a result of *p*-Cresol treatment. Inflammation, hyperplasia, or tumorous lesions were not observed in the urinary bladder.

*p*-Cresol administered by gavage for 13 weeks to rats, at a dosing volume of 5 ml/kg for each dose group of 0, 50, 175, and 600 mg/kg/day, was hepatotoxic, nephrotoxic, and induced a mild anemia; 50 mg/kg/day appeared to be the threshold at which no toxic effects were observed.

A 19-week subchronic feeding study of Thymol (0, 1000, or 10,000 ppm) in weanling rats found no effect in body weights, food intake, liver weights, kidney weights, spleen weights, heart weights, and testis weights.

Rats exposed to 0.05 mg/m<sup>3</sup> Mixed Cresols by inhalation for 90 days exhibited treatment-related effects including CNS excitation, denaturation of lung protein, and decreased body weight gains.

Rats exposed to *o*-Cresol for 4 months (6 h/day) and 2 months (4 or 5 times/week) to a concentration of 9 mg/m<sup>3</sup> had changes in leukocytes, spinal cord smears, nervous activity, liver function, blood effects, clinical signs, and neurological effects. In a similar experiment using guinea pigs, the same concentration produced changes in hemoglobin concentrations and EKG.

Published information on chronic toxicity was only found on PCMC. In a chronic study in which rats were fed  $\leq 10,000$  ppm PCMC for 2 years; the NOEL was 2000 ppm PCMC. No PCMC-related changes in clinical chemistry and hematological parameters were observed and no significant difference in mortality

was observed between test and control animals. At study termination, necropsy findings included kidney deformation and an increase in renal papillary necrosis, cortical tubular dilations, and fibrosis for some of the high dose male rats. Female rats of the mid and high dose groups that died on study had an increased incidence of pituitary adenoma. The NTP concluded that there was little evidence to suggest a significant increase in toxicity with longer exposures of *m*-Cresol, *o*-Cresol, and *p*-Cresol than the 13-week studies as compared to the effects seen with similar doses in the 28-day studies. However, NTP has initiated an oral toxicology/carcinogenesis study on Mixed Cresols.

PCMC (0.05%) produced ocular irritation in the eyes of rabbits, and soft lenses that were stored in solution containing 0.1% PCMC produced severe irritation after a few days. All other Cresols were irritants to the rabbit eye at higher concentrations.

Ingredients in the Sodium *p*-Chloro-*m*-Cresol family, are classified as corrosives (0.79 to 0.98 on a 1.0 scale) and minimal to severe irritants. Primary irritation scores ranged widely from 0.06 to 8.0 on a 8.0 scale. In a dermal irritation study using the trypan blue method, 0.2%, 0.4%, and 0.8% PCMC resulted in maximum irritation scores of 4, 4, and 8, respectively. No primary skin irritation was observed on intact or abraded skin in rabbits exposed to *o*-Cymen-5-ol (0.1% or 1.0%), and 10% *o*-Cymen-5-ol only caused minimal irritation (0.22/4.0) in guinea pigs. *m*-Cresol, *o*-Cresol, or *p*-Cresol were applied dermally to mice for 6 weeks (3× weekly). There was no effect on the skin and hair using 0.5% *m*-Cresol and 0.5% *o*-Cresol. However, repeated application of 0.5% *p*-Cresol had depigmentation and a local caustic, erosive effect on black mice.

Numerous sensitization studies were performed using ingredients in the Sodium *p*-Chloro-*m*-Cresol family. Most positive reactions were seen with higher concentrations of Cresol ingredients. In a GPMT at rechallenge, 7 of 24 guinea pigs pretreated with 2-methylol phenol and 4 of 24 guinea pigs pretreated with 4-methylol phenol reacted (erythema) to 13.1% *o*-Cresol or 13.1% *p*-Cresol, respectively. Weak sensitization effects with 20% Thymol were observed in a GPMT. *o*-Cymen-5-ol was a minimal sensitizer in a GPMT.

No effects were reported in an OET using ≤30% PCMC, 4% *p*-Cresol or 3% Thymol on guinea pigs. *o*-Cymen-5-ol and Carvacrol were positive in an OET; the minimum sensitizing concentration was 3% and the minimum eliciting concentration was 1% for both ingredients. A Draize test was negative using Thymol (0.05 ml, 0.1%) and Carvacrol (0.05 ml, 0.1%). Thymol had no effect on guinea pigs in an FCA test, whereas Carvacrol produced sensitization effects at a total dose of 250 mg. PCMC was a “doubtful” sensitizer; in a CCET, the frequency of sensitization was vehicle dependent.

In rabbits, the *m*-Cresol NOEL for maternal toxicity was 5.0 mg/kg/day and at least 100 mg/kg/day for developmental toxicity, the *o*-Cresol NOEL was 5.0 mg/kg/day for maternal toxicity and 50 mg/kg/day for developmental toxicity, and the *p*-Cresol NOEL was 5.0 mg/kg/day for maternal toxicity and at least 100 mg/kg/day for developmental toxicity.

In rats, the *m*-Cresol NOEL for maternal toxicity was 175 mg/kg/day and at least 450 mg/kg/day for developmental toxicity, the *o*-Cresol NOEL for maternal toxicity was 175 mg/kg/day and was 175 mg/kg/day for developmental toxicity, and the *p*-Cresol NOEL for maternal toxicity was 175 mg/kg/day and 175 mg/kg/day for developmental toxicity.

A two-generation reproduction study using *m*-Cresol on rats was inconclusive regarding developmental toxicity. A two-generation reproduction study using *o*-Cresol or *p*-Cresol on rats resulted in overt toxicity in rats treated with 450 mg/kg/day and produced F<sub>1</sub> offspring that had reduced body weights up to 4 weeks after birth. The *o*-Cresol and *p*-Cresol NOEL for offspring was 175 mg/kg/day.

The NTP evaluated the reproductive toxicity of a mixture of *m*-Cresol and *p*-Cresol using mice under the Continuous Breeding Protocol. NTP concluded that the *m*-Cresol/*p*-Cresol mixture at 1.0% caused minimal adult reproductive and significant post-natal toxicity in the presence of systemic toxicity. The NTP also evaluated the reproductive toxicity of *o*-Cresol. The *o*-Cresol NOAEL was 0.2% for both reproductive and general toxicity in both generations. The data indicate that *o*-Cresol is not a reproductive toxicant to F<sub>0</sub> or F<sub>1</sub> mice under the conditions of this study (up to 1230 mg/kg/day).

In all Ames tests, Cresol ingredients were nongenotoxic. Mixed Cresols were not genotoxic in the fruit fly, two in vitro studies were negative for SCE production and one was positive, positive results were seen with a mammalian forward mutation assay with metabolic activation (but not without), and a transformation assay was positive only at the highest dose level. *m*-Cresol did induce UDS with metabolic activation, but not without. The results of SCE production, chromosome aberration, forward mutation, and dominant lethal mutation assays indicated no genotoxicity. *o*-Cresol was positive in one SCE assay, equivocal in one assay, and was not genotoxic in a third SCE assay. Chromosome aberrations were produced in one study, but cell transformation, forward mutation, UDS, and dominant lethal mutation assays demonstrated no genotoxicity. *p*-Cresol did increase cell transformation in one assay, but cell transformation, SCE production, and dominant lethal mutation assays demonstrated no genotoxicity. Thymol did increase SCE production, UDS, and cell transformation, but did not affect DNA repair in another assay. PCMC induced DNA repair with metabolic activation, but was reduced without.

Thymol (1.20 or 6.00 g/kg) did not induce primary lung tumors in mice.

No skin tumors were found in mice when one drop (25 μl) of each *m*-Cresol, *o*-Cresol, or *p*-Cresol was placed on a shaved abdomen twice weekly at 20% (in benzene) for 12 weeks.

*m*-Cresol, *o*-Cresol, and *p*-Cresol have the same order of activity as phenol in terms of skin tumor promotion. At the 2.5-mg phenol dose, mice treated with 10% phenol (in benzene) developed their first carcinomas at 19 weeks, at 26 weeks 50% of mice had carcinomas, and at 42 weeks 73% of mice had carcinomas. Few skin tumors developed on the mice treated with DMBA or

phenol alone; there was only one fibrosarcoma at 58 weeks in mice treated with phenol alone.

*p*-Cresol is a microbial amino acid metabolite that has shown weak promoting activity in animal studies. The findings indicated that the “endogenous metabolites [*p*-Cresol] do not contribute significantly to the development of human bladder cancer.”

In the trypan blue exclusion assay, antitumor effects were observed for Thymol and Carvacrol.

Exposure to cresol can occur through air, water, or food. The PEL for Cresol isomers are 5 ppm or 22 mg/m<sup>3</sup> in air and Cresol isomers have a skin designation. All 14 countries listed in ILP Occupational Exposure Limits for Airborne Toxic Substances as of 1991 have set an workplace concentration of 5 ppm for TWA exposure for all Cresol isomers. NIOSH recommended a TLV of 10 mg/m<sup>3</sup>, which the USEPA used to derive a chronic inhalation exposure limit of 7.143 mg/day (assuming a human breathing volume of 10 m<sup>3</sup>/day, a 5 day work week, and using an uncertainty factor of 10 to protect more sensitive individuals in a population).

In clinical studies, patch testing with 2% PCMC may produce irritant reactions, particularly in people with multiple patch test reactions, that are misinterpreted as allergic responses. *o*-Cresol, *p*-Cresol, Thymol, and Carvacrol caused no dermal irritation at 4% each. Thymol had no dermal effects at 1% to 5% and *o*-Cymen-5-ol had no effect at 0.1% to 1.0%.

In two predictive patch tests, initiation with up to 20% PCMC followed by challenge with 5% PCMC did not produce a sensitization reaction in 31 or 252 subjects. In several provocative patch tests, some reactions to PCMC were usually observed. Cross-reactivity between PCMC and chloroxylenol has been observed, but only when the initial sensitization was to chloroxylenol.

Two of 81 patients (already sensitized to textile dyes) were sensitized to 2% *m*-Cresol. Twenty-five subjects were tested with 4% *o*-Cresol, no sensitization effects noted. Out of 10 hand dermatitis patients, 4 patients tested positive at 81% *o*-Cresol, 3 tested positive at 8.1%, and no effects were observed at 0.81% *o*-Cresol. Out of 10 hand dermatitis patients, 1 patient tested positive at 81% *p*-Cresol and there were no effects at 8.1% *p*-Cresol.

A human RIPT using 99 subjects to assess the skin sensitization of 0.1% *o*-Cymen-5-ol found no evidence of skin sensitization. A maximization test conducted on 27 men determined 1% *o*-Cymen-5-ol did not produce any reactions that could be considered irritant or allergic in nature.

Eighty-four stomatological staff were patch tested with 1% Thymol; only 1 patient reported a positive reaction to 1% Thymol. From 1967 to 1970, 0 of 290 patients with eczematous dermatitis had a reaction to 1% Thymol (in petrolatum). In a 48-h patch test in 50 normal people, the highest tolerated concentration of Thymol was 5% (in petrolatum). Twenty perfume-sensitive male and female patients were given standard 48-h patch tests using 1% Thymol (in petrolatum). One “perfume-sensitive” patient reacted to Thymol.

One of two contact dermatitis patients patch tested with 1% Carvacrol elicited a positive response. Three of 28 patients allergic to perfumes and sweet-smelling constituents elicited a positive reaction when patch tested with 2% Carvacrol. Thirty-one subjects and 33 subjects were dermally exposed to 4% and 8% Carvacrol (in petrolatum), respectively, with no adverse reactions. Two of 179 patients patch tested with 5% Carvacrol in petrolatum had positive reactions, but those may have been false positives due to excited skin syndrome.

Twenty-five women were induced via six occlusive patches (1% *p*-Cresol in a hydrophilic ointment) each followed by 3-MED irradiation. Challenge was 10 days later via an occlusive patch followed by 4 joules/cm<sup>2</sup>. No photoallergic reactions were noted. There were 11 allergic contact sensitivity reactions and 7 primary irritation reactions.

*o*-Cresol is a respiratory irritant in humans. Eight of 10 subjects exposed to “brief exposure” to a concentrated aerosol of 6 mg/m<sup>3</sup> *o*-Cresol complained of dryness, nasal constriction, and throat irritation.

The Agency for Toxic Substances and Disease Registry (ATSDR) developed a list of hazardous substances that included *m*-Cresol, *o*-Cresol, and *p*-Cresol. The ATSDR minimal risk levels (MRLs) were 0.05 mg/kg/day for acute oral exposure with a respiratory end point.

## DISCUSSION

The Panel acknowledged that the NTP has initiated a toxicology/carcinogenesis study on Mixed Cresols. The Panel expects to consider those data when they are available.

The CIR Expert Panel noted some of these ingredients may increase the penetration of other cosmetic ingredients. Cosmetic formulators should take this into consideration when using these ingredients.

The Panel acknowledged the minimal amount of data on UV absorption. However, the ingredients in this report absorb light in the UVC to the low UVB range. This is consistent with the one clinical test in which 25 women tested negative in a photoallergy study. Therefore, the risk of phototoxicity/photoallergy was considered low.

The Panel also noted that PCMC, Cresol, and Thymol have international restrictions. PCMC is approved for use by members of the European Union as a preservative at a maximum concentration of 0.2%; however, it may be added to cosmetic products at a concentration other than that specified for a preservative for other specific purposes apparent from the presentation of the product. PCMC is prohibited for use in products intended to come in contact with mucous membranes. The Japanese MHLW have restrictions on Cresol, Thymol, and PCMC when used as a preservative. As preservatives, Cresol (rinse-off and leave-on products), Thymol (rinse-off and leave-on products), and PCMC (all products) are limited to 0.010, 0.05, and 0.50 g per 100 g, respectively. Cresol and Thymol are not permitted for use in cosmetics that are applied

on mucous membranes (except Thymol when limited to the mouth).

Although acknowledging these restrictions, the CIR Expert Panel concluded that the toxic effects of these ingredients are observed at doses higher than would be available from cosmetics. In order to ensure that these ingredients are used at a level that would not cause any of the toxicity noted in this report, however, the Panel believes that an upper concentration limit is needed. In particular, and apropos of the discussion below, data are available to demonstrate that *m*-Cresol and *o*-Cresol when tested at 0.5% do not cause chemical leukoderma (skin depigmentation). It may be that Sodium *p*-Chloro-*m*-Cresol, *p*-Chloro-*m*-Cresol, Chlorothymol, *m*-Cresol, *o*-Cresol, Isopropyl Cresols, Thymol, *o*-Cymen-5-ol, and Carvacrol do not cause chemical leukoderma at concentrations higher than 0.5%, but data are not available to support that possibility. Therefore, a concentration limitation of 0.5% was chosen to ensure the absence of a chemical leukoderma effect.

*p*-Cresol is demonstrated to cause depigmentation of hair and possibly the skin. In addition, there is concern that *p*-Cresol may be a strong allergen. There was insufficient data for the Panel to determine a safe use level that would not sensitize or produce chemical leukoderma for *p*-Cresol and Mixed Cresols (which contains *p*-Cresol). Therefore, the Panel considered that the available data are insufficient to support the safety of these two ingredients in cosmetics. Studies are needed which would demonstrate no chemical leukoderma at concentrations of use of *p*-Cresol and Mixed Cresols, or would demonstrate a dose response from which a safe concentration could be derived.

## CONCLUSION

The CIR Expert Panel concludes Sodium *p*-Chloro-*m*-Cresol, *p*-Chloro-*m*-Cresol, Chlorothymol, *m*-Cresol, *o*-Cresol, Isopropyl Cresols, Thymol, *o*-Cymen-5-ol, and Carvacrol are safe at concentrations up to 0.5% in cosmetics; however, the available data are insufficient to support the safety of *p*-Cresol and Mixed Cresols for use in cosmetic products.

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